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MODELLING THE OBSERVED VELOCITY OF OUR GALAXY RELATIVE TO THE COSMIC MICROWAVE BACKGROUND

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The aim of this work is to model the observed velocity of our galaxy relative to the cosmic microwave background that was recently discovered by Smoot et al. A homogenous and isotropic, flat, relativistic two fluid cosmological model is considered in which two separate fluids act as the source of the gravitational field. In this model one fluid is a comoving radiative perfect fluid modelling the cosmic microwave background and the second a non-comoving imperfect fluid modelling the observed material content of the universe. The model that is obtained is represented as a solution of Einstein's equations and the laws of thermodynamics in which all the physical quantities occurring in the solution are suitably well behaved. In addition, the model is in good agreement with current observations.

Le but de ce travail est de modeler la vitesse observée de notre galaxie par rapport aux microondes cosmiques de fond découvertes récemment par Smoot et al. On considère un modèle cosmologique qui est homogène, isotropique, plat et relativistique composé de deux fluides distincts qui sont la source du champ gravitationnel. Dans ce modèle, un des fluides est un fluide parfait, radiatif et codéplacent qui représente les microondes cosmiques de fond et l'autre fluide représente le contenu matériel observé de l'univers. Le modèle ainsi obtenu est représenté sous forme d'une solution aux équations d'Einstein et les lois de la thermodynamique dans laquelle toutes les quantités physiques qui s'y trouvent se comportent normalement. En plus, le modèle s'accorde bien avec les observations actuelles.

Introduction

It is currently believed that except for very early times the evolution of the universe is adequately described by "an FRW model". The conventional wisdom is that the universe evolved initially from a radiation-like state to a matter-like universe at later times.

The original Friedmann-Robertson-Walker (FRW) models were solutions of Einstein's equations in which the source of the gravitational field was either a comoving radiative perfect fluid or a comoving matter perfect fluid. Radiation models were to govern the evolution of the universe for early times while models such as the Einstein de Sitter model were supposed to be applicable at more advanced times. Later, in order to obtain a single model governing the evolution of the universe, known radiation models and known matter models were matched continuously at the time when the energy densities of the radiation and matter fields were equivalent (see, for example, Gamow [1956] or Jacobs [1967]).

Many authors (McIntosh [1968a] or [1968b]) were stimulated to investigate FRW models which include both radiation matter fields for all times by the discovery in 1965 of the 2.7°K isotropic cosmic microwave background (Penzias and Wilson [1965] and Roll and Wilkinson [1966]), which was presumed to be a remnant of the "primeval fireball". In these models two (separate) comoving perfect fluids are assumed to

act as the source of the gravitational field. (A review of the standard theory of two fluid cosmological models is given in Coley and Tupper [1986]).

Recently it was discovered (Smoot et al. [1977]) that there is an observed motion of our galaxy relative to the cosmic microwave background, which has stimulated our present interest in attempting to model this observed feature of the universe. That is, we shall seek a model in which there are two cosmological fluids, the first a comoving fluid representing the background radiation field and the second a matter fluid constituting the observed material content of the universe which is non-comoving so that there is a relative motion between the two fluids.

It is observed that both the cosmic microwave background and the observed matter are approximately isotropic and homogeneous. Consequently, we wish to study models in which isotropy and homogeneity is preserved, namely FRW models. In particular we shall consider zero curvature FRW models here, for which the metric is given by

$$ds^2 = -c^2dt^2 + R^2(t)(dx^2 + dy^2 + dz^2), \tag{1}$$

in "axial" coordinates, where t is the cosmic time and R the expansion factor. Our assumed model is therefore an FRW model in which there are two fluids present which act as the source for the gravitational field (as represented by (1)), a comoving radiation field and a tilting matter field. We note that this is only possible if the matter field is represented by an imperfect fluid (Coley and Tupper [1983], [1984], and [1986]).

It has been established that FRW models can be interpreted as solutions of Einstein's equations for a variety of different sources (Coley and Tupper [1983] and [1984]). From the comments above we can see how the study of FRW models due to increasingly more sophisticated matter distributions is associated with progressive discoveries concerning the nature of the universe.

Preliminaries

Henceforward we shall be seeking a two fluid FRW model of the following description. We shall assume that the first fluid is a comoving, perfect fluid with the (black body) radiative equations of state

$$\rho_r = aT_r^4, \quad \frac{p_r}{c^2} = \frac{1}{3}\rho_r \tag{2}$$

where ρ_r, p_r and T_r are the density, pressure and temperature of the radiation and $a = 7.57 \times 10^{-15}$ erg cm^{-3}deg^{-4} is Stefan's constant. The comoving 4-velocity of the radiation is represented by $v_i = (-c,0,0,0)$. This fluid will model the observed cosmic microwave background. The second fluid will be taken to be a non-comoving imperfect fluid with density ρ_m, pressure p_m, temperature T, 4-velocity u^i, shear tensor σ^{ij}, shear viscosity coefficient η and heat conduction vector q^i. This second fluid will model the observed matter in the universe.

In this configuration Einstein's equations take on the form

$$\frac{c^4}{8\pi G}G_{ij} = \frac{\rho_r}{3}(4v_iv_j + c^2g_{ij}) + (\rho_m + \frac{1}{c^2}p_m)u_iu_j + p_mg_{ij} - 2\eta\sigma_{ij} + q_iu_j + q_ju_i. \tag{3}$$

The matter is assumed to be moving axially relative to the comoving radiation, thus modelling the observed relative velocity between the centre of our galaxy and the cosmic microwave background. Consequently, we shall take u_i to be of the form

$$u_i = (-\alpha, 0, 0, \beta R) \tag{4}$$

where $\alpha^2-\beta^2 = c^2$, and α and β are assumed to be functions of t alone. We also assume that

$$q_i = \frac{Q}{c}(\beta, 0, 0, -\alpha R) \tag{5}$$

so that $q_i u^i = 0$ and $Q^2 \equiv q_i q^i$.

Using Eqns. (1), (3) and (4), Eqns. (2) become

$$\rho_m = \frac{1}{c^2}\left\{\frac{1}{8\pi G}\left[\frac{\dot{R}^2}{R^2}(3\alpha^2-\beta^2) - \frac{2\beta^2\ddot{R}}{R}\right] - \frac{\rho_r}{3}(3\alpha^2+\beta^2)\right\} \tag{6}$$

$$3p_m = \frac{1}{8\pi G}\left\{\frac{\dot{R}^2}{R^2}(5\beta^2 - 3\alpha^2) - \frac{2\ddot{R}}{R}(3\alpha^2 - 2\beta^2)\right\} - \frac{\rho_r}{3}(3\alpha^2+\beta^2) \tag{7}$$

$$\frac{\eta\dot{\alpha}}{c^3} = \frac{-\beta^2}{2c^2}\left\{\frac{1}{8\pi G}\left[\frac{2\ddot{R}^2}{R^2} - \frac{2R}{R}\right] - \frac{4}{3}\rho_r\right\} \tag{8}$$

$$Q = \frac{1}{c}\left\{\frac{1}{8\pi G}\left[\frac{2\dot{R}^2}{R^2} - \frac{2\ddot{R}}{R}\right] - \frac{4}{3}\rho_r\right\}\alpha\beta \tag{9}$$

where a dot denotes differentiation with respect to t. The right-hand sides of Eqns. (6) and (7) are positive which always ensures that the terms in braces on the right-hand sides of Eqns. (8) and (9) are positive, so that Q is the same sign as β and η is non-negative if and only if

$$\dot{\alpha} \leq 0\,. \tag{10}$$

We shall not take T_r and T to be equal in the model. This means that the two fluids will not be in thermodynamical equilibrium throughout the history of the universe. This is precisely what is to be expected if an imperfect fluid with non-zero heat conduction vector is present. We note that it is presently believed that the current temperature of the "matter" in the universe is about four times higher than that of the cosmic microwave background.

It will be assumed that the physical quantities associated with the imperfect fluid satisfy the following set of thermodynamical laws.

(i) Baryon conservation law: $(nu^\mu)_{;\mu} = 0$. This equation can be integrated so that $n = n_0 R^{-3}\alpha^{-1}$. n is the particle density and n_0 a constant.

(ii) Gibbs' relation: $Td(Sn^{-1}) = d(\rho_m n^{-1}) + p_m d(n^{-1})$. We note that if the "integrating factor" T is a function of t alone, a solution of the Gibbs'relation, which can now be thought of as an equation to determine the entropy density S, is guaranteed.

(iii) Temperature gradient law: $q^\mu = -\kappa c^{-6}(g^{\mu\nu} + u^\mu u^\nu)(c^2 T_{,\nu} + T u_{\nu;\pi} u^\pi)$.We note that the condition that the thermal conductivity κ is non-negative ensures that entropy production is also non-negative. In the situation under investigation we find that the temperature gradient law reduces to a single equation, which can be used to determine κ. It can be shown that the condition for $\kappa \geq 0$ then reduces to

$$\frac{\dot{T}}{T} + \frac{\dot{\beta}}{\beta} + \frac{\dot{R}}{R} \geq 0. \tag{11}$$

We note that although the two fluids in the model are not in thermal equilibrium, deviations from thermodynamical equilibrium are not so large that the applicability of the assumed laws of thermodynamics is brought into question.

In addition, we demand that the energy conditions $\rho_r > 0, \rho_m > 0, \rho_m - c^{-2}p_m \geq 0$ and $p_m \geq 0$ are all satisfied in the model. We also expect T to be a decreasing function of t. Finally, we impose the following "boundary conditions" on the model:

$$\begin{aligned} \alpha &\to c \quad \text{as} \quad t \to \infty \\ \alpha &\to \infty \quad \text{as} \quad t \to 0 \end{aligned} \qquad (12)$$

The Model

In our model we shall take R(t) to be of the form

$$R(t) = t^{1/2}(1 + \ell t^{3/5})^{5/18}, \qquad (13)$$

where ℓ is a positive constant such that $\ell t^{3/5}$ is dimensionless. With this choice of R(t), which is a monotonically increasing function of time, we have that $R(t) \to t^{1/2}$ as $t \to 0$, so that initially the universe was in a pure radiation state (initially even the matter field was in a radiation-like state), and $R(t) \to t^{2/3}$ as $t \to \infty$, so that the model evolves towards the Einstein de Sitter universe (in which the matter field will completely dominate). We remark that the specification of R(t) above (and α and T below) is effectively equivalent to specifying the equations of state for the fluid; for example a relationship results between ρ_m and p_m (which we note is allowed to vary with time). The question of whether such a "choice" of equations of state is physically acceptable is answered when the solution is obtained and all the physical quantities appearing in the resulting model are found to be suitably well behaved. Finally, from $8\pi G R^2 \rho_r \equiv -3(2\ddot{R}R + \dot{R}^2)$ we find that

$$\rho_r = \frac{3}{32\pi G}(t^{-2})(1+\ell t^{3/5})^{-2}(1+\frac{8}{15}\ell t^{3/5}). \qquad (14)$$

We assume that α and β are of the form

$$\frac{\alpha}{c} = \frac{1 + ht^{-q}}{(1 + 2ht^{-q})^{1/2}}, \quad \frac{\beta}{c} = \frac{ht^{-q}}{(1 + 2ht^{-q})^{1/2}}, \qquad (15)$$

where h and q are positive constants. With this choice of α we note that conditions (12) are satisfied, and a brief calculation yields $\dot{\alpha} < 0$ guaranteeing $\eta > 0$ for all t (from Eqn. (10)). We also assume T is of the form

$$T = T_0 t^{-b} R^{-p} \alpha^s, \qquad (16)$$

where b, p and s are positive constants.

Condition (11), ensuring κ is non-negative, takes on the form of an algebraic inequality involving t and the arbitrary parameters h and ℓ and b, p, q and s. It can be shown that this inequality will be satisfied for all t, and all h and ℓ, if either of the following two sets of inequalities are satisfied:

$$s \leq 1 \quad \text{and} \quad \frac{1}{2} - \frac{p}{2} - b - q \geq 0 \qquad (17a)$$

$$s > 1 \quad \text{and} \quad 1 - p - 2b - q - sq \geq 0. \qquad (17b)$$

There will be various solutions depending on the desired behaviour of physical quantities (such as T) as $t \to 0$ or $t \to \infty$. Here, we shall make the following assumptions. First, since $R(t) \to t^{1/2}$ as $t \to 0$, we shall assume that $\rho_m^{-1} T^4 \to$ constant as $t \to 0$, which implies that $7/5 + q - 4b - 2p - 2qs = 0$. Second, we shall assume that as $t \to \infty$, $\rho_m^{-1} T^a \to$ constant where $a \leq 4$, which implies that $b/2 + p/3 \geq 1/4$. Finally, for simplicity we shall assume that $b = 0$ and $s \leq 1$ so that the conditions to be satisfied become:

$$p \geq \frac{3}{4} \ , \ \frac{7}{5} + q - 2p - 2qs = 0, \ \ 1 - p - 2q \geq 0. \tag{18}$$

From these conditions we find that $p \leq 19/25$. This suggests two straightforward solutions in which $p = 3/4$ or $p = 19/25$. Let us concentrate on the model in which $p = 19/25$; a complete solution of (18) is then

$$p = \frac{19}{25}, q = \frac{3}{25}, \ s = 0, \ b = 0. \tag{19}$$

In this case $T = T_0 R^{-19/25}$ and $\rho_m \sim T^{75/19}$ for large t. The end conditions for this model have been described above. Using Eqns. (6), (7), (8), (9) and the aforementioned laws of thermodynamics the full solution (representing the model) is given by

$$\rho_m = \frac{\ell}{5\pi G}(1 + \ell t^{3/5})^{-2}(1 + \frac{5}{6}\ell t^{3/5})(1 + 2ht^{-3/25})^{-1}(1 + ht^{-3/25})^2 t^{-7/5}$$

$$\frac{3p_m}{c^2} = \frac{\ell h^2}{5\pi G}(1 + \ell t^{3/5})^{-2}(1 + \frac{5}{6}\ell t^{3/5})(1 + 2ht^{-3/25})^{-1} t^{-41/25}$$

$$\eta = \frac{5c^2\ell}{6\pi G}(1 + \ell t^{3/5})^{-2}(1 + \frac{5}{6}\ell t^{3/5})(1 + 2ht^{-3/25})^{1/2} t^{-2/5} \tag{20}$$

$$\frac{Q}{c} = \frac{\ell h}{5\pi G}(1 + \ell t^{3/5})^{-2}(1 + \frac{5}{6}\ell t^{3/5})(1 + 2ht^{-3/25})^{-1}(1 + ht^{-3/25}) t^{-38/25}$$

$$n = \frac{n_0}{c}(1 + \ell t^{3/5})^{-5/6}(1 + 2ht^{-3/25})^{1/2}(1 + ht^{-3/25}) t^{-3/2}$$

$$\kappa = \frac{5c^2}{T_0 \pi G}(1 + \ell t^{3/5})^{-71/90}(1 + \frac{5}{6}\ell t^{3/5})(1 + 2ht^{-3/25})^{1/2}(1 + ht^{-3/25}) t^{-31/50}$$

$$\times [1 + \frac{3h}{\ell} t^{-18/25} + 5ht^{-3/25}]^{-1}.$$

We note that in this model η and κ are always positive. In addition, ρ_m and p_m are always positive and monotonically decreasing, and $3p_m/c^2\rho_m \to 1$ as $t \to 0$ and $3p_m/c^2\rho_m \to 0$ as $t \to \infty$. The model is physically acceptable from a theoretical point of view since Einstein's equations and the laws of thermodynamics are satisfied, and all physical quantities appearing in the model are suitably well-behaved. The observational predictions of this model will be discussed below.

Observations

We let the subscript zero denote the present time. All numerical values will be calculated to three significant places only. We shall assume that the value of the

arbitrary positive constant ℓ is given by $\ell = 1.06 \times 10^{-7}$ (see Coley [1985]). Based upon a Hubble parameter $H_0 = 55$ km sec^{-1} Mpc^{-1} we find that $t_0^* \equiv H_0^{-1} = 5.67 \times 10^{17}$ secs, so that from the definition of H_0 and Eqn. (13) we find that $t_0 = 3.78 \times 10^{17}$ secs (the age of the universe). We note that $\ell t_0^{3/5} = 3.73 \times 10^3$.

We shall assume that the present velocity of our galaxy relative to the cosmic microwave background is three hundred kilometers per second (Smoot et al. [1977]), so that from Eqns. (15) we find that $ht_0^{-3/25} = 1.00 \times 10^{-3}$, which fixes h as $h = 1.29 \times 10^{-1}$.

From Eqn. (20) we find that $\rho_{m,0} = 5.57 \times 10^{-30}$ g cm^{-3}. From Eqn. (14) we find that $\rho_{r,0} = 4.47 \times 10^{-34}$ g cm^{-3} and hence from $\rho_r = aT_r^4$ we find that $T_{r,0} = 2.70°$K. In addition, Eqn. (20) yields $c^{-2} p_{m,0} = 1.86 \times 10^{-36}$ g cm^{-3}, and finally we obtain $p_{m,0}/c^2\rho_{m,0} = 3.33 \times 10^{-7}$.

Let t_e be the time when $\rho_r = \rho_m$. From Eqns. (14) and (20) we then obtain a quadratic equation in $\ell t_e^{3/5}$. Taking the positive root, and using the established valued of ℓ, we find that $t_e = 1.01 \times 10^{11}$ secs. The rate of energy transfer per unit volume from radiation to matter E_m (as defined by Davidson [1962]) is found to be

$$E_m = \frac{c^2\ell}{50\pi G} (t^{-12/5}) (1 + \ell t^{3/5})^{-3} (1 - \frac{1}{6} \ell t^{3/5}). \qquad (21)$$

Since $E_m \neq 0$ in this model, there will be interaction (that is, energy transfer) between the two fluids. For small t, E_m is positive (as desired). For larger values of t, such as at present, it is believed that there is a conversion or net rate of gain of energy per unit volume from radiation to matter (ie., $E_{m,0} < 0$) due to the nuclear burning of stars in galaxies. In our model, from Eqn. (21) we find that $E_{m,0} = -7.16 \times 10^{-32}$ erg cm^{-3} sec^{-1}.

Comparing the above with current numerical estimates in the literature (Coley and Tupper [1986]) we see that the predictions of the model are in excellent agreement with actual observations. The model is consequently a physically acceptable cosmological model. Moreover, our model is also able to predict a relative velocity of the galaxy with respect to the cosmic microwave background of 300 km sec^{-1}. We conclude by remarking that through the model described above we have achieved our goal of modelling the observed motion of our galaxy relative to the cosmic microwave background.

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References

Coley, A.A. 1985. Cosmological models with both radiation and matter. *Can. J. Phys.* 63: 1199-1200.

Coley, A.A. and **Tupper, B.O.J.** 1983. Zero-curvature Friedmann-Robertson-Walker models as exact viscous magnetohydrodynamic cosmologies. *Astrophys. J. 271:* 1-18.

Coley, A.A. and **Tupper, B.O.J.** 1984. Imperfect fluid cosmologies with thermodynamics: some exact solutions. *Astrophys. J. 280:* 26-33.

Coley, A.A. and **Tupper, B.O.J.** 1986. Two-fluid cosmological models. *J. Math. Phys. 27:* 406-416.

Davidson, W. 1962. The cosmological implications of the recent counts of radio sources. II: an evolutionary model. *Mon. Not. R. Astron. Soc. 124:* 79-93.

Gamow, G. 1956. *Vistas in Astronomy* II, Ed. A. Beer, Pergamon Press, London.

Jacobs, K.C. 1967. Friedmann cosmological model with both radiation and matter. *Nature 215: 1156-1157.*

McIntosh, C.B.G. 1968a. Cosmological methods with both radiation and matter. *Mon. Not. R. Astron. Soc. 138:* 423-436.

McIntosh, C.B.G. 1968b. Relativistic cosmological models with both radiation and matter. Mon. Not. R. Astron. Soc. 140: 461-472.

Penzias, A.A. and **Wilson, R.W.** 1965. A measurement of excess antenna temperature at 4080 Mc/s. *Astrophys. J. 142:* 419-421.

Roll, P.G. and **Wilkinson, D.T.** 1966. Cosmic background radiation at 3.2 cm - support for black-body radiation. *Phys. Rev. Lett. 16:* 405-407.

Smoot, G.F., Gorenstein, M.V. and **Muller, R.A.** 1977. Detection of anisotropy in the cosmic blackbody radiation. *Phys. Rev. Lett. 39:* 898-901.

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PYROLYSIS/MAGNETIC SEPARATION: A PROCESS FOR REDUCING THE SULPHUR CONTENT OF EASTERN CANADIAN COALS*

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Elements of a process are described, whereby the high sulphur coals of eastern Canada may be converted into a low sulphur product suitable for coal-slurry fuels. The process envisaged includes thermal/magnetic benefication followed by aggregative flotation. On a laboratory scale the optimum conditions for these steps include vacuum pyrolysis at 475°C, and wet magnetic separation at a field strength of 1.6×10^{-5} A.m^{-1}. A favourable tar yield also occurred at about 475°C for chars exposed to vacuum pyrolysis.

Les étapes d'un processus par lequel les charbons en teneur élevé de soufre provenant de l'est du Canada peuvent être convertis en un combustible à teneur réduite de soufre sont décrites. Le processus envisagé inclu la bénéficiation thermale/magnétique suivi d'une flotation agrégative. A l'échelle du laboratoire les conditions optimales pour ces étapes comprennent la pyrolyse sous vide à 475°C, et la séparation magnétique à une intensité de champ de 1.6×10^{-5} A.m^{-1}. Un rendement favorable en goudron est aussi obtenu à 475°C pour les charbons soumis à la pyrolyse sous vide.

Introduction

Between 1981 and 1985[1] an advisory board, the Committee on Atlantic Coal, with representation from the users and suppliers of coal, and Federal and Provincial governments, consistently identified the reduction of the sulphur content of coal destined to be burned in eastern Canadian electric power stations as a priority target for research.

The literature on the reduction of sulphur in coal is voluminous; useful reviews are those of Moore (1982), and Attia (1985). When sulphur is removed before the coal is burned it may be by either physical or chemical means. Physical methods usually remove only that sulphur that is associated with the intrusive mineral matter of the coal; examples are the many processes generally named "coal washing." Chemical cleaning may in addition remove some or all of the sulphur which is associated with the carbonaceous matrix itself. This paper describes work carried out to investigate

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[1] During this time the National Research Council of Canada received funds from the National Energy Program which were mainly devoted to contract research in institutions in eastern Canada. Some of this work has been published in the Technical Report Series of the Atlantic Research Laboratory. Copies of these reports may be obtained from the Librarian of the Laboratory.

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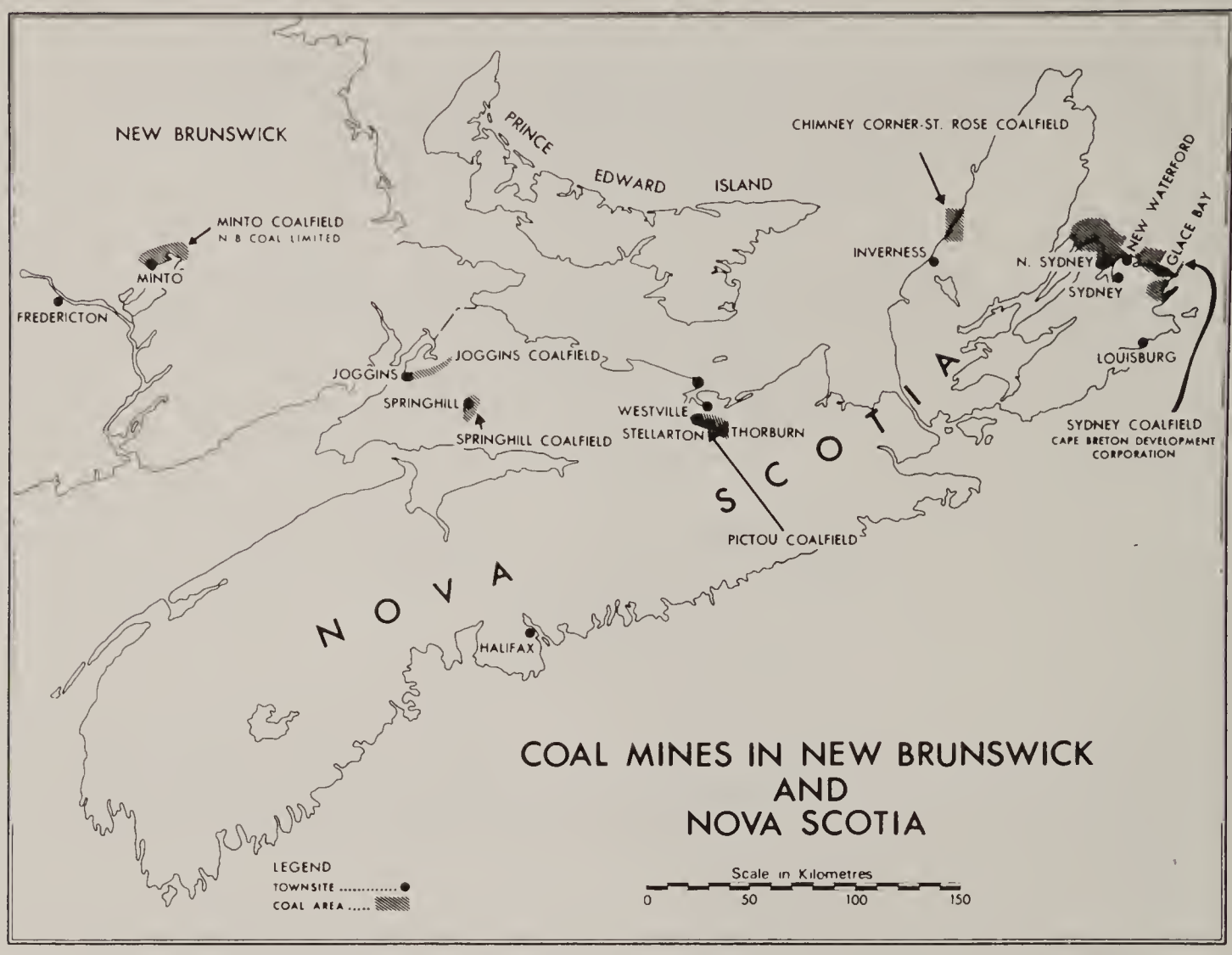

Fig 1 Coal mines in New Brunswick and Nova Scotia.

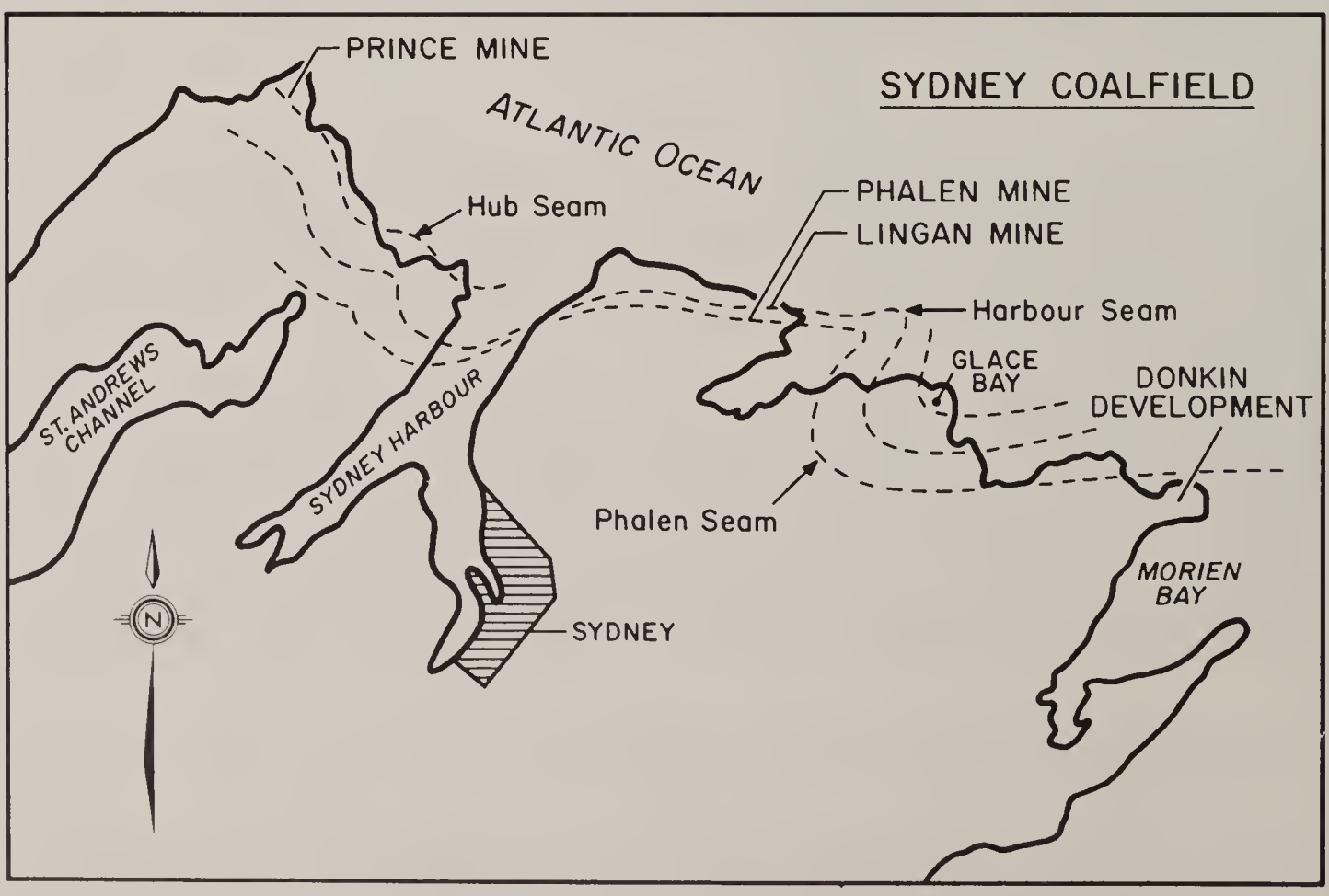

Fig 2 Map of Sydney coal field, showing outcrops and mines.

aspects of a method to remove sulphur before the coal is burned, having elements of both physical and chemical cleaning. It was based on the fact that mild pyrolysis of coal is known to change pyritic sulphur to a strongly magnetic form (Liu and Lin 1976) amenable to removal by a magnetic process. New results on magnetic beneficiation are given, and they are put in context by including some of the results of peripheral investigations.

In eastern Canada coal is found in the provinces of New Brunswick and Nova Scotia, as shown in Fig 1 and Fig 2. Currently the most active mining sites are the Minto coalfield in New Brunswick and the Sydney coalfield in Nova Scotia.

In New Brunswick the coal is high in ash and sulphur (Bonnell, Janke and Romaniuk 1983). In the Sydney coalfield (Fig 2) the bulk of the 2 billion tonnes of thermal coal potentially available can be divided loosely into two groups — about two-thirds having 3.9% sulphur and one-third containing 5.3% sulphur. This sulphur is present in three forms: organic, pyrite and sulphate. Organic sulphur (about 33% in high-sulphur coal) occurs as an integral part of the carbonaceous matrix and cannot be removed by physical means. Pyritic sulphur (about 66%) is found mainly as iron pyrite (FeS_2) outside of the carbon matrix; sulphate (trace) also occurs in this intrusive mineral fraction.

Stirling (1984) has studied the mineralogy of coals from the Sydney, Westville and Minto coalfields and concluded that the maturity of crystallization of pyrite increased in the order Sydney → Westville → Minto. The presence of the allotrope marcasite in the Sydney region coincided with this immaturity of crystallization. The grain size of pyrite particles in typical eastern coals also was reported in this study. For the Prince Colliery coal of the Sydney coalfield, 60% of the pyrite occurs in grains under 100 μ, and 20% is smaller than 20 μ.

Physical beneficiation of these coals for sulphur, using conventional float-sink washing methods based on the relative densities of coal and pyrite is not efficient. Coal from the Prince Colliery, with about 4% sulphur, 65% of which was pyrite, was processed through the coal cleaning test facility of the Electric Power Research Institute at Homer City, PA. Whereas sulphur reduction was achieved it was at the expense of carbon recovery. For example, when the specific gravity of the separation fluid was adjusted to 1.67 the energy recovery was 89%, but the sulphur had been reduced only from 4% to 3.8%. When the specific gravity was lowered to 1.3 the sulphur was reduced to 1.8%, but the energy recovery fell to only 60% (Mikhlin 1987).

The beneficiation of coal containing finely dispersed sulphur requires fine grinding of the coal, the carbon being recovered by some method which depends upon its

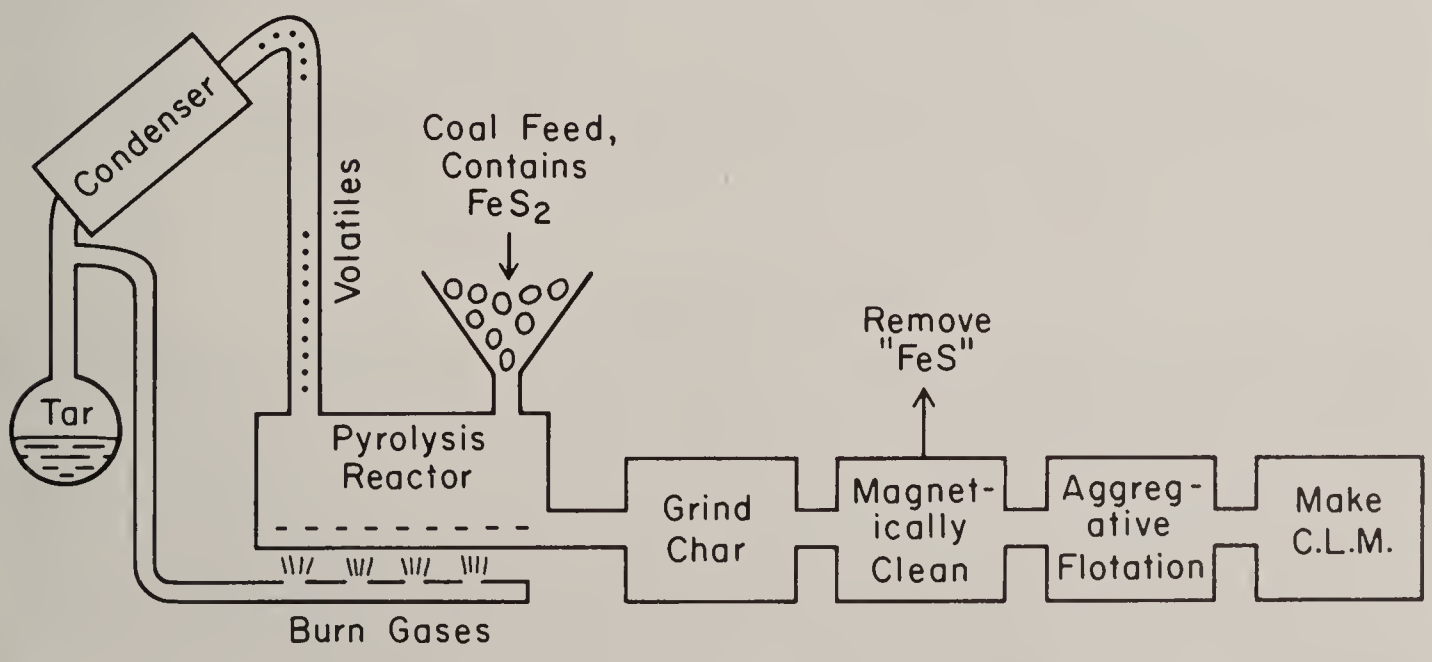

Fig 3 Conceptual process combining pyrolysis, beneficiation, coal-water mixtures.

hydrophobic nature. In the oil agglomeration method sulphur is not rejected efficiently from the carbon because pyrite is not strongly hydrophilic. A process developed to alter the nature of the surface of pyrite by adsorbing on it a hydrophilic layer of Thiobacillus ferrooxidans bateria (McCready 1984) worked well for Minto coal, with good pyrite rejection at acceptable carbon recoveries, but the same was not true of Prince coal from the Sydney coalfield.

The work presented here is based on the premise that some method other than conventional washing will be required to remove the pyrite from eastern thermal coals. Magnetic separation is one such method. Coal is weakly diamagnetic while pyrite is weakly paramagnetic, and separation of these two requires high magnetic field gradients. However, when coal is heated to a temperature variously reported as being between 440 and 580°C, its pyrite decomposes to a pyrrhotite of composition near Fe_7S_8 (Jagadeesh and Seehra 1981). Depending upon the stoichiometry, this pyrrhotite may have a magnetic susceptibility about 7200 times that of pyrite. With such a change a separation based upon a magnetic process should be efficient.

A conceptual process sharing some of the features proposed by Fine, Lowry, Power and Geiger (1976) is shown in Fig 3. Coal is pyrolysed in a reactor to the correct temperature for formation of the most magnetic species of pyrrhotite. A vacuum process is envisaged because separate work (Roy and deCaumia 1984) showed that the quality of the coal tars produced is enhanced. The gases emitted would be used to fuel the process, and the tar liquids recovered for sale. The char is ground and the pyrrhotite removed magnetically, probably by a wet process. The char slurry would then be beneficiated for ash by aggregative oil flotation (AlTaweel and Wojcik 1983) possibly using some of the pyrolysis liquids as the collecting agent, and finally would be made into a clean coal-water-oil liquid fuel. Some parts of this conceptual process have been investigated and will be discussed.

Experimental

Pyrolysis experiments were carried out in a 1 kg capacity stirred tank reactor, equipped with temperature programming, and capable of being operated at pressures from 50 torr to atmospheric. All the gas was collected and analysed for amount and composition, the liquids were trapped and weighed, and the char was weighed and analysed. A complete description of this reactor, and associated analytical methods, has been given previously (Hirajima, Chan, Whiteway, Stefanski and Stewart 1985). Other methods used and described in previous publications are scanning electron microscopy (Cleyle, Caley, Stewart and Whiteway 1984) and Mössbauer spectroscopy (Stewart, Whiteway, Cleyle and Caley 1986).

The magnetic beneficiation of the above chars has been studied using a susceptibility balance and an isodynamic separator arranged for wet operation.

Results and Discussion

A typical plot of the emission of H_2S from 2% sulphur coal of the Sydney field, pyrolysed at atmospheric pressure, is shown in Fig 4 (curve B) (Whiteway and Galvez 1978). The maximum emission rate at 475°C is due to decomposition of FeS_2. The smaller emission at 425°C is probably decomposition of organic sulphur. Added pyrite of size 200 microns, mixed with the coal, did not decompose below 560°C (Fig 4, curve A). Thus we might expect to see some variation in the decomposition temperature of pyrite depending upon the size of individual grains and their intimacy of admixture with the carbon matrix.

Fig 5 shows a microprobe analysis of actual grains of sulphide in a coal of 4% sulphur pyrolysed at different temperatures (Cleyle, Caley, Stewart and Whiteway

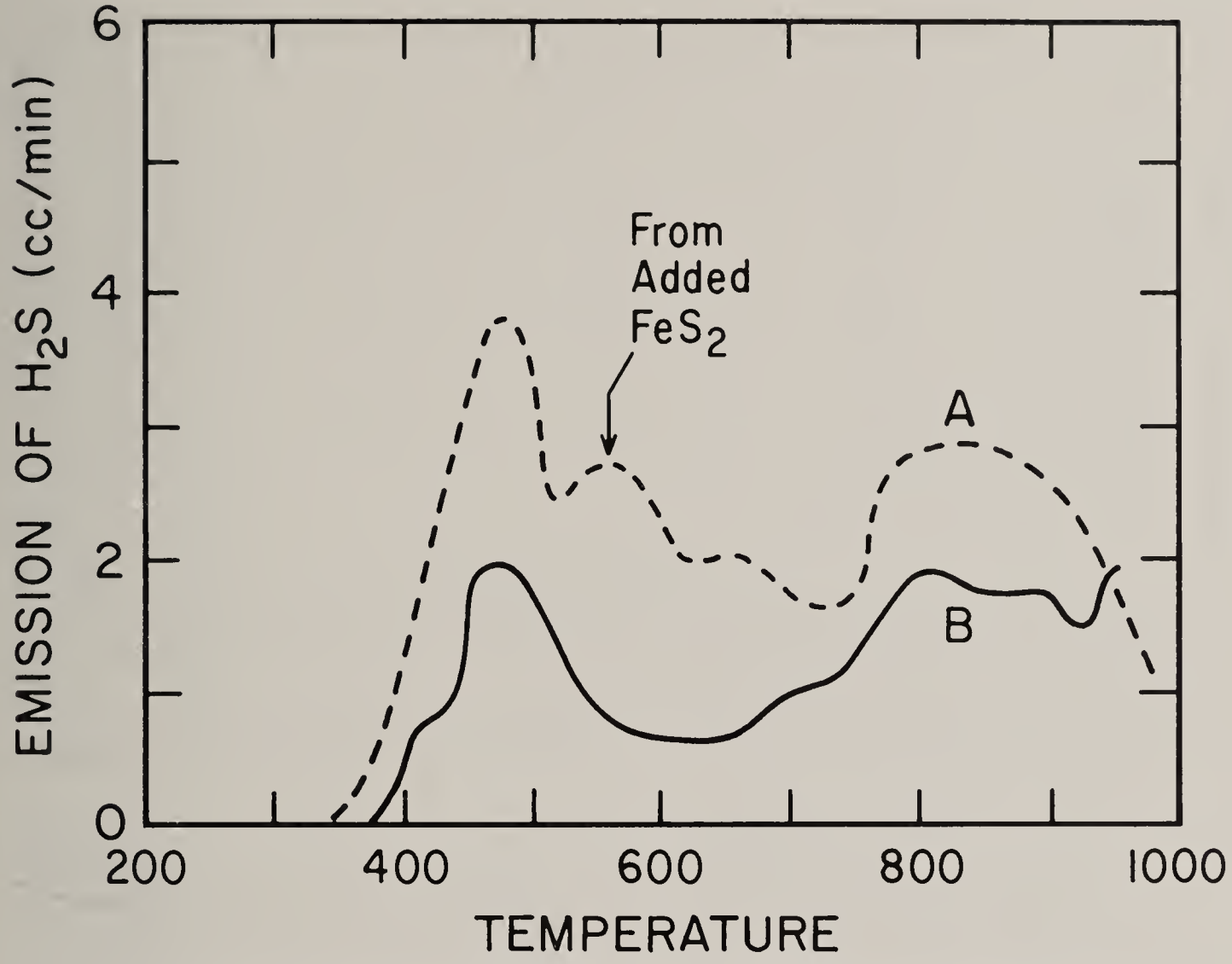

Fig 4 Emission spectra of H_2S from coal from Harbour Seam. (A) with extra FeS_2 added. (B) normal coal (Whiteway and Galvez 1978).

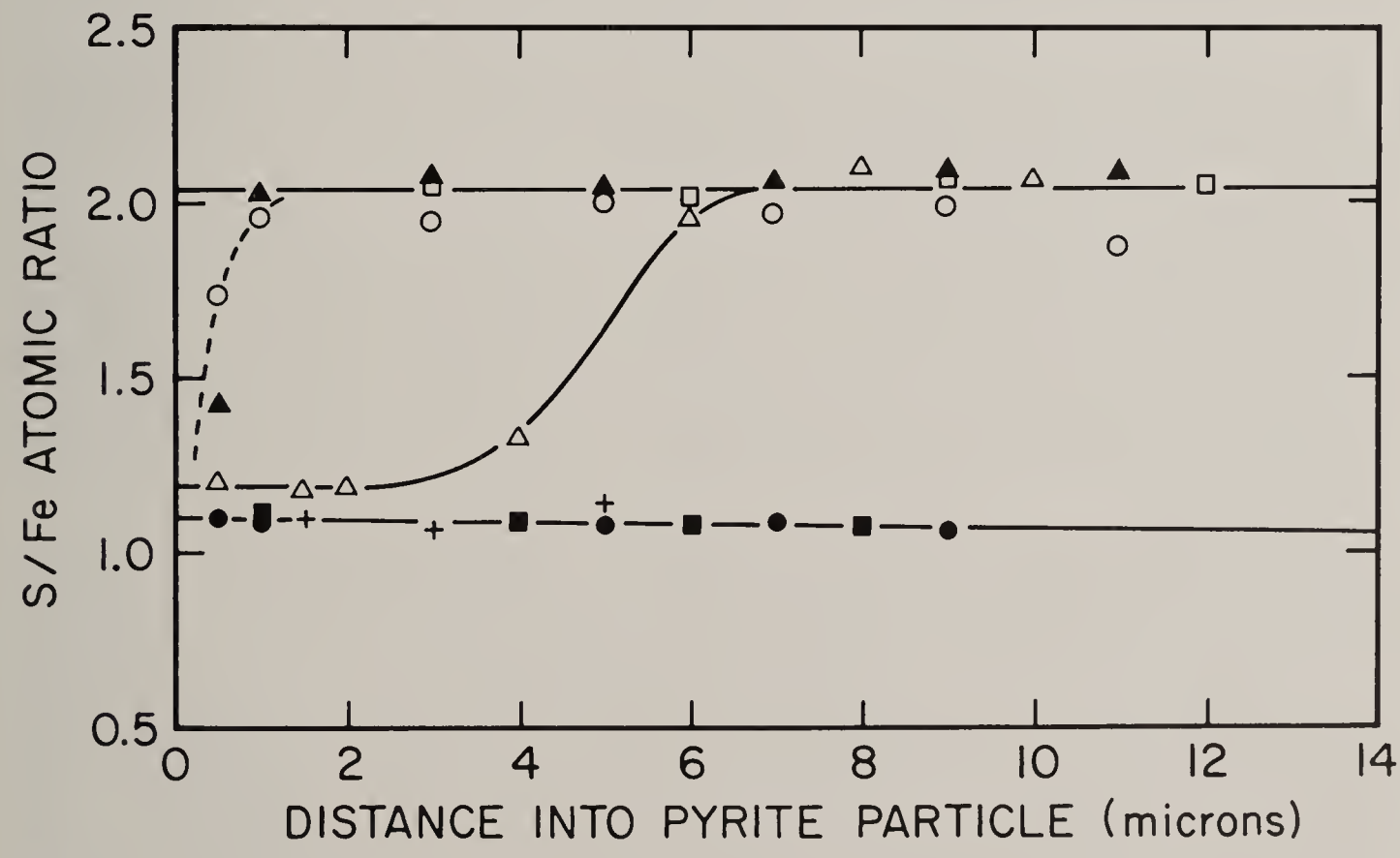

Fig 5 Plot of S/Fe atomic ratios versus distance from the coal/pyrite interface, moving into the centre of the pyrite particle, for a number of pyrolysis temperatures. Pyrolysis times 15-20 hours. □, untreated coal; O, 400°C; ▲, 500°C; Δ, 550°C; ●, 600°C; ■, 650°C; +, 700°C. (From Cleyle, Caley, Stewart and Whiteway 1984).

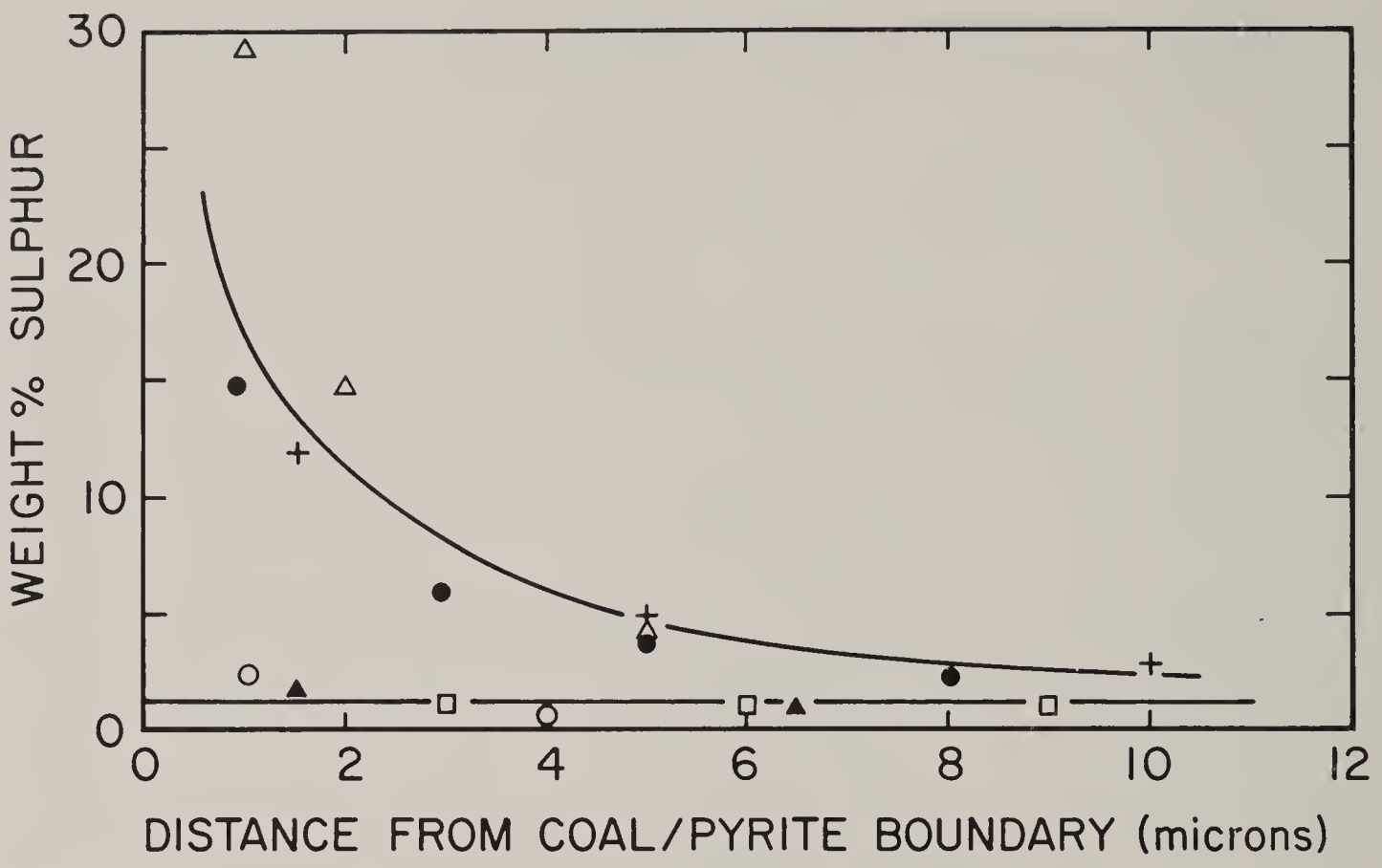

Fig 6 Plot of weight % sulphur in the carbonaceous matrix around the pyrite particles versus distance from the coal/pyrite interface, moving into the matrix. Pyrolysis time 15-20 hours. □, untreated coal; O, 400°C; ▲, 500°C; Δ, 550°C; ●, 600°C; +, 700°C. (From Cleyle, Caley, Stewart and Whiteway 1984).

1984). The grains were about 20 μ in diameter. No decomposition was seen at 500°C, while at 600°C and above all the original pyrite had changed to pyrrhotite of non-stoichiometric composition, with slightly more sulphur than iron. At a temperature of 550°C a grain was found that had partially decomposed, the non-stoichiometric sulphide at the surface appeared to have a higher S/Fe ratio than that of the completely decomposed grains. Fig 6 shows the sulphur content in the coal matrix just around grains of sulphide. Until the decomposition temperature for pyrite had been reached the sulphur content was low, and presumably represented the organic sulphur. After the pyrite had decomposed the coal matrix showed a considerably higher sulphur content close to the pyrrhotite. It is evident that considerable sulphur can be trapped in this manner by the coal; other work suggests that it becomes strongly bound to the carbon matrix as a high-temperature organic form (Attar 1978).

Several points may be made from this work bearing upon the possibility of magnetic beneficiation of chars. The extent to which individual grains of pyrite decompose to pyrrhotite will depend upon the temperature and time of pyrolysis, but at a given temperature may also depend upon the grain size and the exact chemical nature of the coal. In addition the stoichiometry and the magnetic susceptibility may depend upon the extent of decomposition. It may not be necessary for grains to be completely decomposed to achieve a useful level of susceptibility. A very magnetic shell of pyrrhotite may be able to carry with it an unreacted core of pyrite. In addition the trapping of sulphur as "organic sulphur" in the coal matrix may not be deleterious if this portion of the coal, upon grinding, is attached to the magnetic sulphide.

The following pyrolysis results are typical for coal from the Prince Colliery of the Sydney coalfield, of 30% volatile matter, 15% ash, and 4.8% sulphur (the latter 0.6 sulphate, 2.7 pyritic and 1.5 organic).

Table I Distribution of Products From Pyrolysis of Prince Coal

T (°C)	P (torr)	CHAR	GAS (weight fraction)	TAR
392	50	0.854	0.019	0.127
386	500	0.862	0.016	0.122
470	50	0.766	0.038	0.196
474	500	0.772	0.054	0.174
580	50	0.692	0.077	0.231
565	500	0.730	0.081	0.189

Table II Characteristics of Chars From Prince Coal

T (°C)	P (torr)	Ash	V.M. (dry basis weight %)	F.C.	Total S (%)
392	50	17.0	20.5	62.5	5.49
386	500	16.8	21.2	62.1	5.53
470	50	18.6	14.9	66.5	4.97
474	500	18.7	12.9	68.4	4.54
580	50	19.5	8.5	72.0	3.76
565	500	19.0	8.4	72.6	3.97
ROM Coal	15.0	30.3	54.7	4.78	

Table III Forms of Sulphur of Chars from Prince Coal

T (°C)	P (torr)	Gms Sulphur/100 Gms original Coal Total	SO_4	Pyritic	Sulfide	Organic
ROM Coal	4.78	.56	2.68	-	1.54	
392	50	4.69	.26	2.68	-	1.75
386	500	4.77	.24	2.78	-	1.74
470	50	4.00	.18	2.18	.29	1.36
474	500	3.50	.19	0.79	1.08	1.45
580	50	2.60	.03	.03	1.51	1.02
565	500	2.90	.05	.03	1.51	1.30

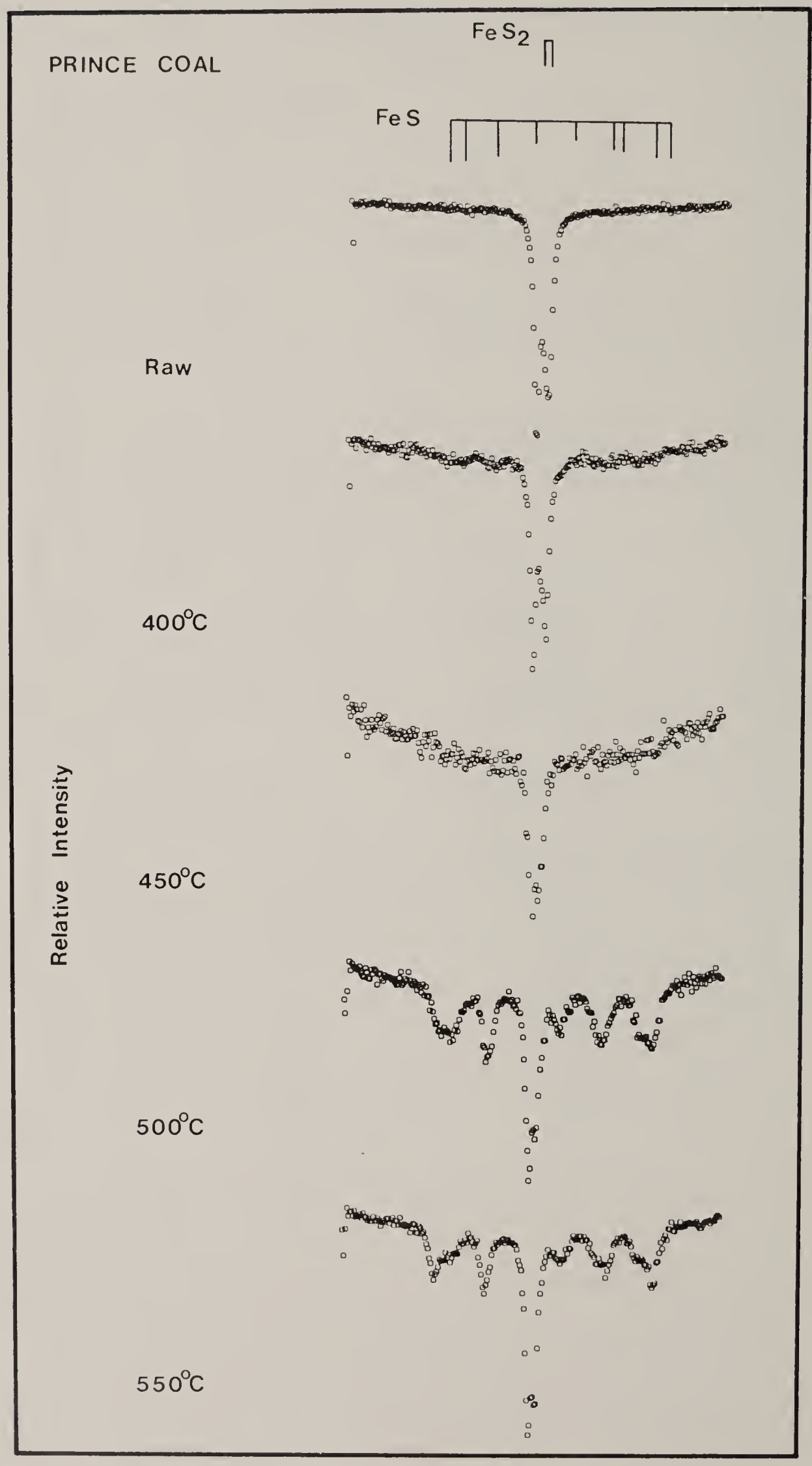

Fig 7 Mössbauer spectra of coal as it is pyrolysed. (From Stewart, Whiteway, Cleyle and Caley 1986).

Table I gives the distribution of the three products: char, liquid and gas. The extent of devolatilization goes up as the temperature is increased, and the ratio of liquids to gases increases as the pressure is decreased. Table II gives some of the characteristics of these chars. Chars made at 470°C still retain 13-15% volatile matter, and so should be readily combustible. Table III gives the distribution of the forms of sulphur in the chars (in g sulphur/100 g coal). For this analysis the sulphide was inferred from the drop in pyrite, assuming that the sulphide formed was Fe_7S_8. It was also assumed that the sulphate was reduced to sulphite, not sulphide, during the pyrolysis. These results show that the sulphate was reduced, the pyrite disappeared, pyrrhotite was produced, and there was a loss of organic sulphur. The runs at 470°C show that the decomposition temperature for pyrite was affected by pressure; apparently under vacuum the reactive gases were removed more rapidly from the coal matrix and were not as available for reaction with pyrite.

Fig 7 shows Mössbauer spectra for Prince coal pyrolysed in the temperature range 400-550°C (Stewart, Whiteway, Cleyle and Caley 1986). The decomposition of pyrite to yield pyrrhotite is clearly shown by the relative amounts of the species FeS_2 and FeS

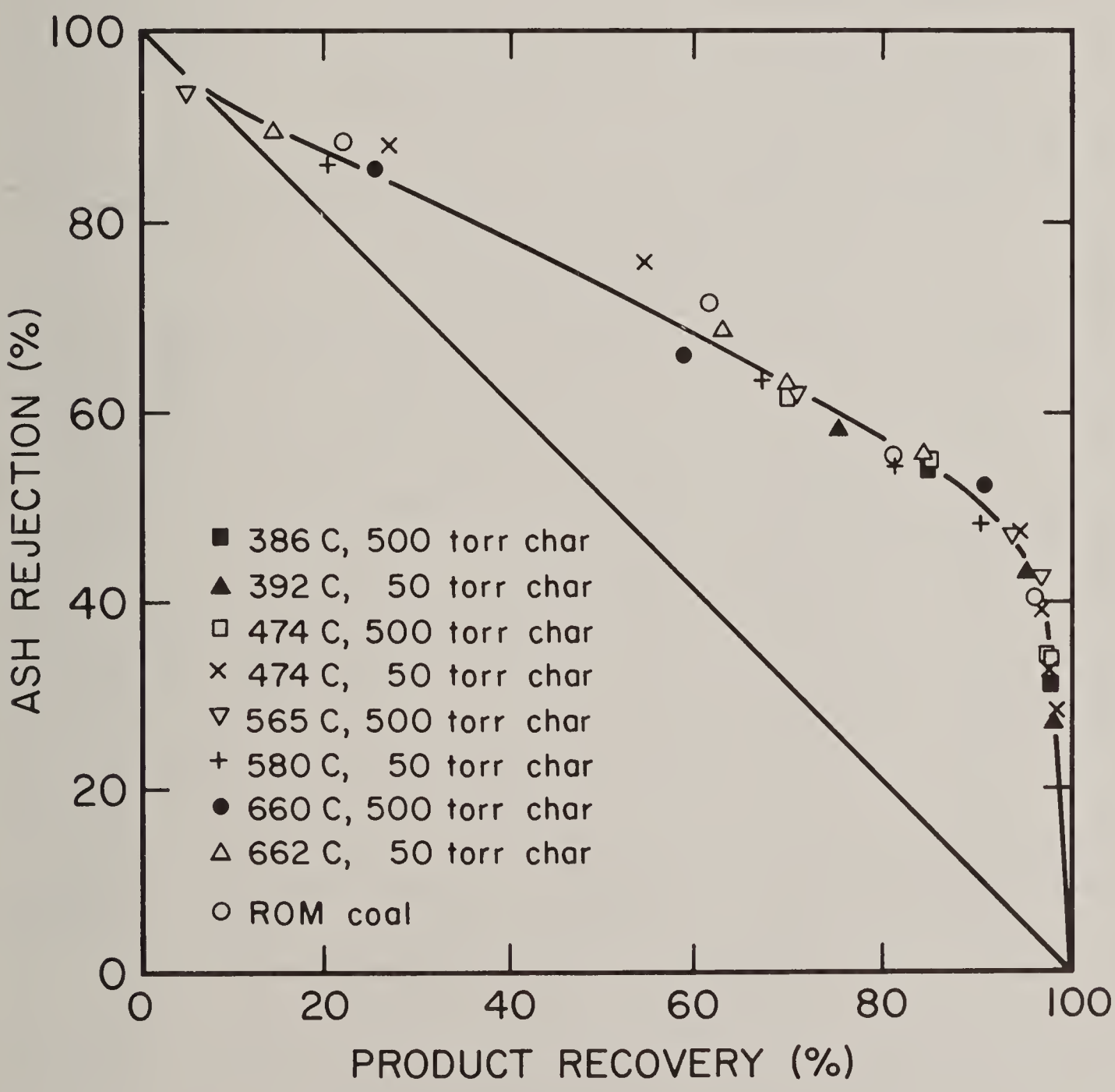

Fig 8 Beneficiation of char for ash by agglomerative flotation. (From Hirajima, Chan, Whiteway, Stefanski and Stewart 1987).

as the temperature was increased. However, there was a wide temperature range over which the two forms coexisted; for example, some pyrrhotite had already formed at 400°C, while pyrrhotite was still present at 500°C. Recent work (Caley, Whiteway and Stewart 1988) largely explains this wide range, by showing that the morphology of pyrite inclusions is an important factor in their decomposition temperature.

While the overall conceptual process would have an additional magnetic cleaning step for sulphur, we have examined the chars for washability with an agglomerative flotation column, using kerosene as an agglomerating agent and methyl isobutyl carbinol as frothing agent (Hirajima, Chan, Whiteway and Stefanski 1985). Fig 8 shows the ash rejection for vacuum and atmospheric pressure chars, and Fig 9 shows the sulphur rejection for vacuum chars. There was little difference in the ash rejection characteristics of all the chars and the parent coal. In terms of sulphur rejection the higher temperature chars showed little benefication.

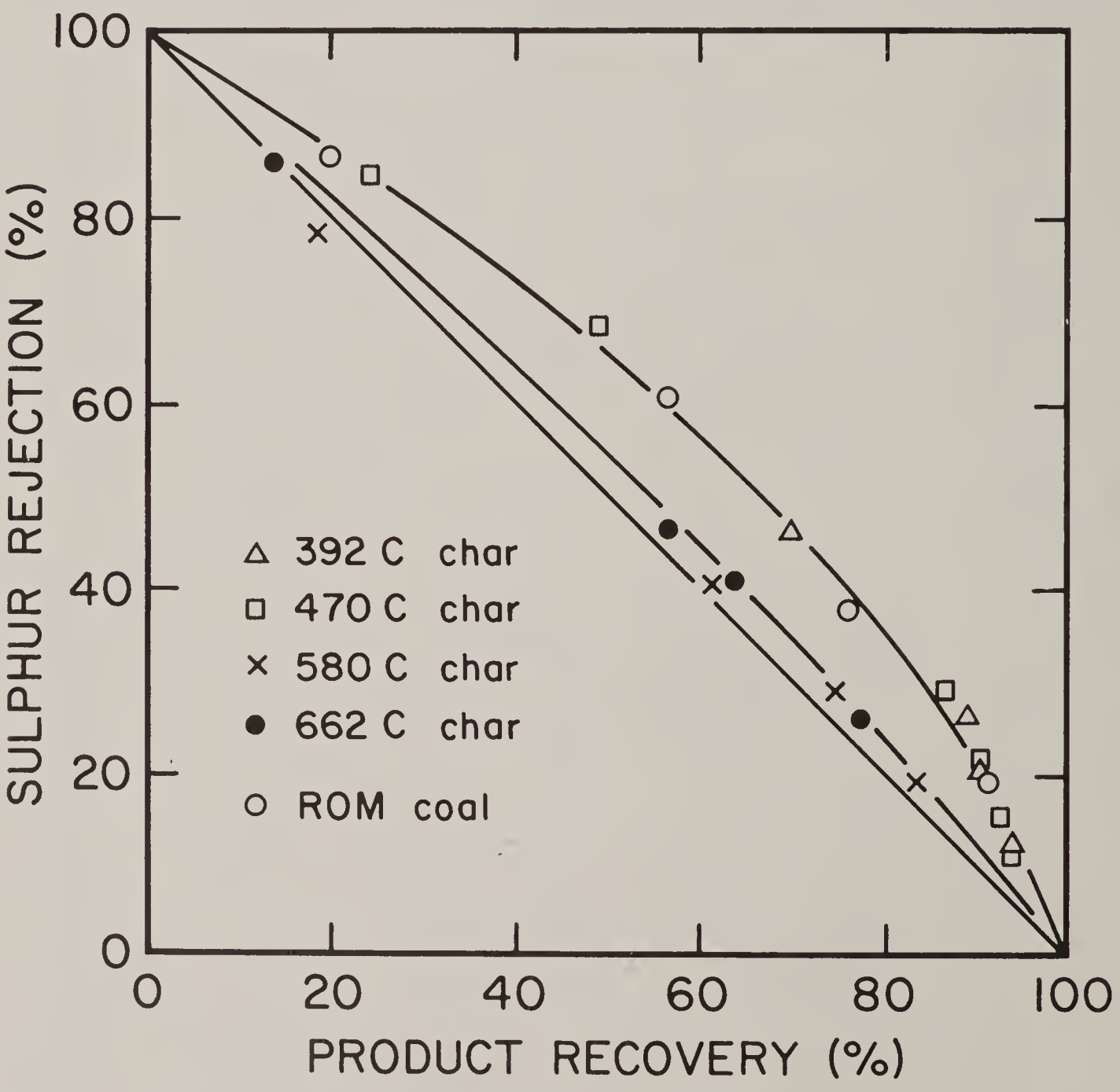

Fig 9 Beneficiation of char for sulphur by agglomerative flotation. (From Hirajima, Chan, Whiteway, Stefanski and Stewart 1987).

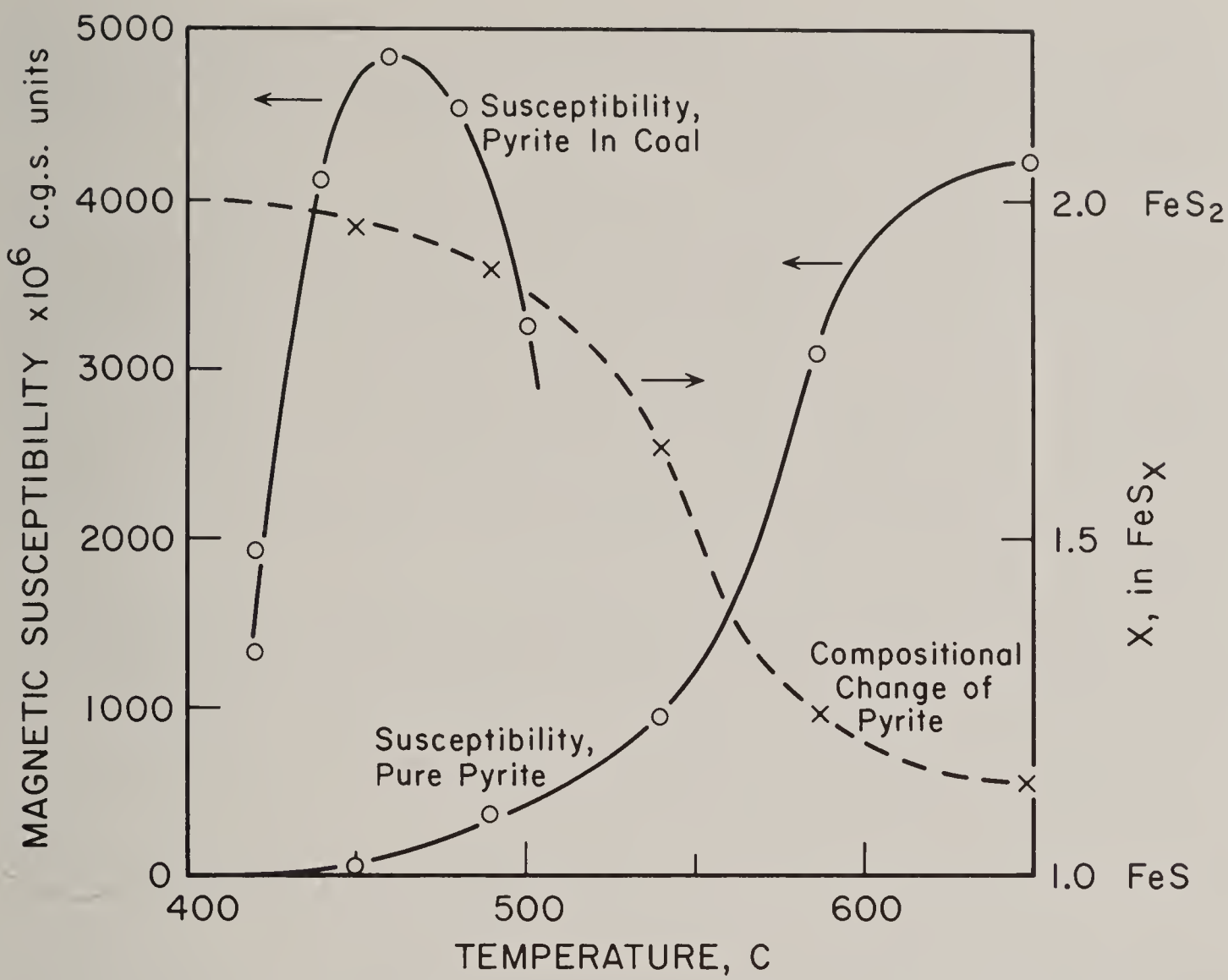

Fig 10 Magnetic susceptibility of pyrite, field strength 2000 Oersted.

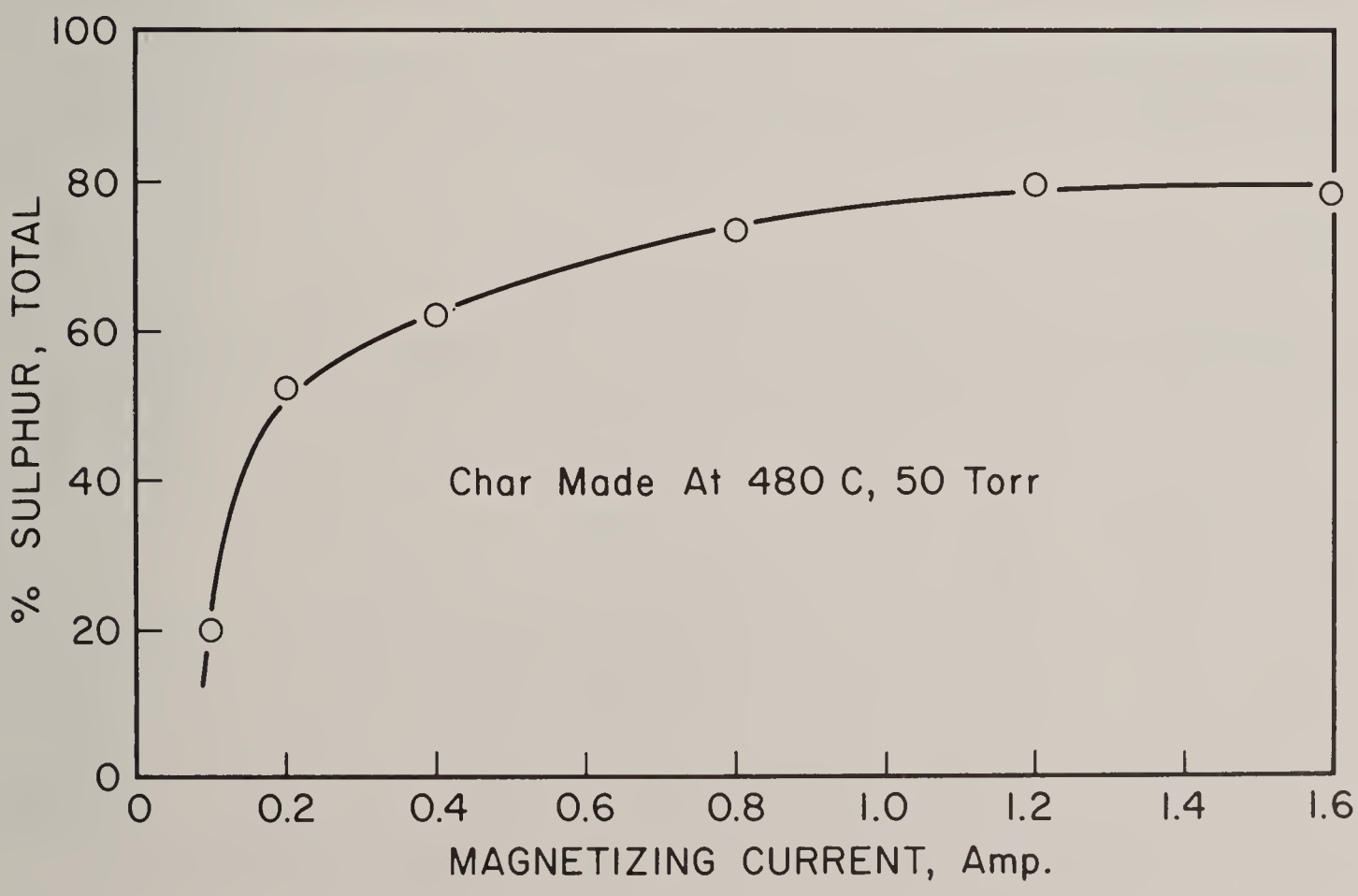

Fig 11 Percentage of original char sulphur in the magnetic fraction, as a function of magnetizing current.

Fig 10 shows how the magnetic susceptibility of pure pyrite increases as it decomposes to pyrrhotite, coming to a maximum at around 650°C. By comparison the susceptibility of finely ground pyrite added to coal (2 gm FeS_2 in 10 gms coal) peaked at about 470°.

Chars made at 480°C and 50 torr were ground and put through the magnetic separator. The sulphur collected in the magnetic fraction varied with the field strength, Fig 11. Some carbon was lost to the magnetic fraction. Fig 12 shows the recovery of carbonaceous product in the non-magnetic fraction, on an ash-free basis, as a function of magnet current. Fig 13 shows the effect of pyrolysis temperature over a range of pressures, on the fraction of total sulphur that was collected in the magnetic fraction. These results indicate that a current of 0.4 amps and a pyrolysis temperature of 475°C gave both a high sulphur rejection and a good product recovery. Of added benefit is the fact that this temperature is in the range already recommended (Roy and deCoumia 1984) from the standpoint of optimization of yield of coal tars.

The economic feasibility of the process envisaged has not been studied. In 1988, at a time of low world oil prices, capital investment in coal projects is not at a high level. In time, the acid rain problem will become greatly reduced with the introduction of

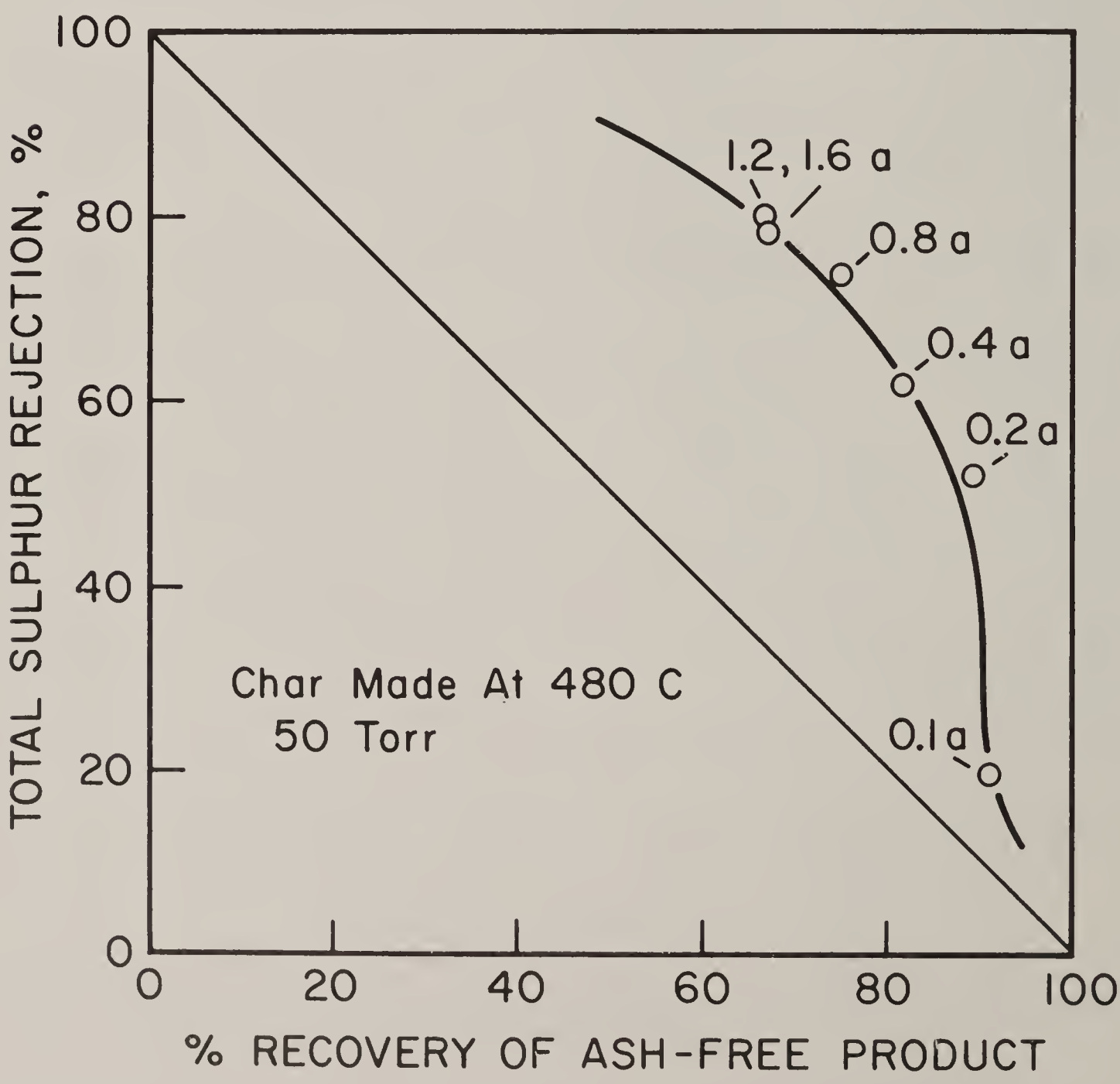

Fig 12 Beneficiation of char for sulphur: effect of magnetizing current.

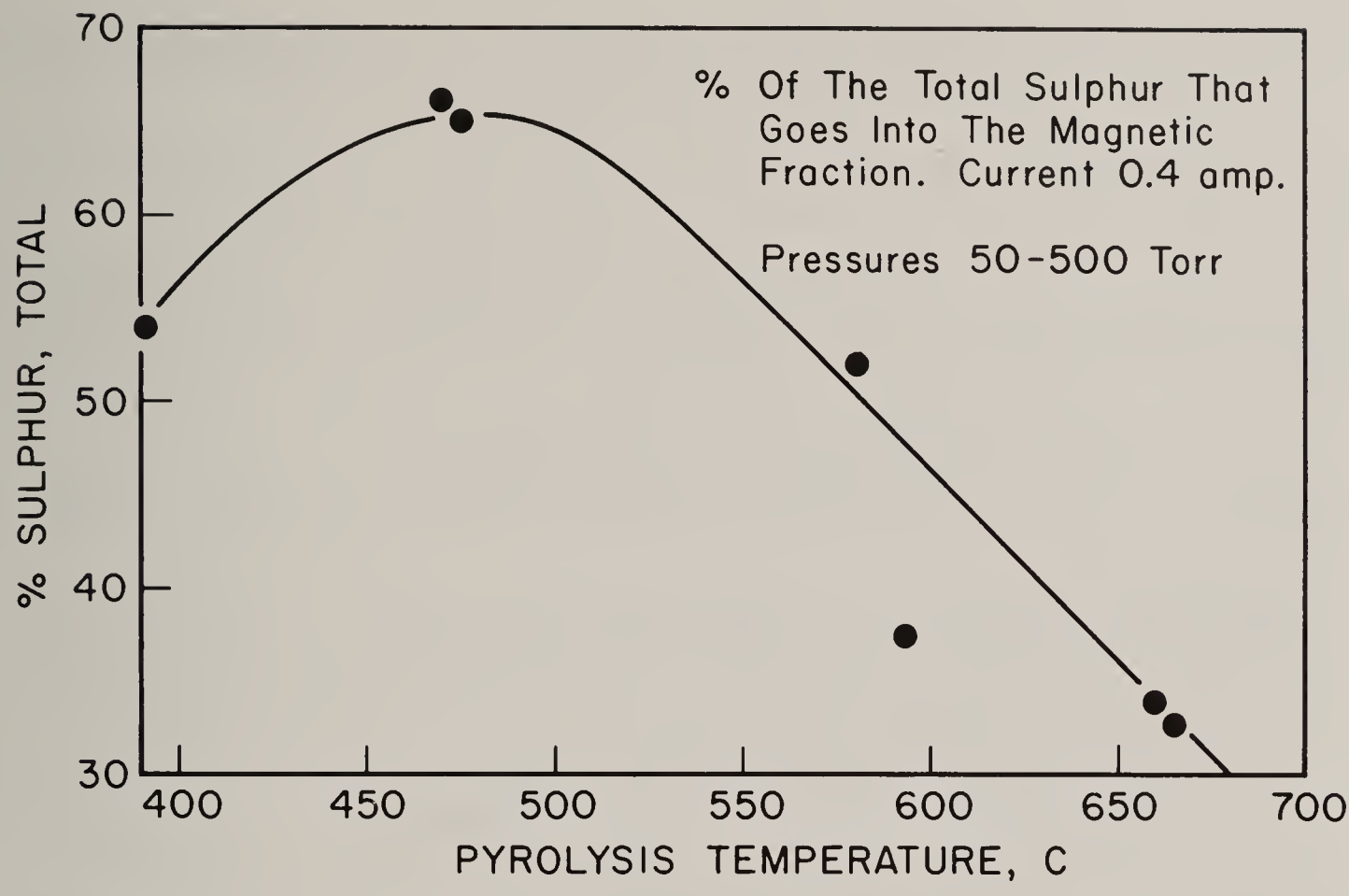

Fig 13 Percentage of original char sulphur in the magnetic fraction, as a function of temperature of pyrolysis.

advanced recirculating fluid-bed combustors. Therefore the process discussed here may be more relevant to the production of oil-like fuels, the coal-water slurries. It is noteable that the liquid hydrocarbons given off in the pyrolysis step can be used in the oil-agglomeration step, leading to economies in the process, and reintroducing a volatile component which will aid combustion. Coal-water slurries should find a continuing market with small industrial users who are not in a position to handle bulk coal.

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References

AlTaweel, A. and **Wojcik, J.** 1983. *"Improvement in Aggregative Flotation Techniques."* Atlantic Regional Laboratory Technical Report No. 45.

Attar, A. 1978. "Sulfur Reactions in Coal Pyrolysis and Hydrogenation." *Coal Processing Tech.* 4:26-34.

Attia, Y.A. 1985. *"Processing and Utilization of High Sulfur Coals,"* Elsevier. 787 p.

Bonnell, G.W., Janke, L.C. and **Romaniuk, A.S.** 1983. *"Analysis Directory of Canadian Commerial Coals — Supplement No. 5."* CANMET Report 84-1E.1.

Caley, W.F., Whiteway, S.G. and **Stewart, I.** 1988. "Variation of the Reactivity of FeS_2 in Eastern Canadian Coal as a Function of Morphology." Coal Preparation: accepted for publication.
Cleyle, P.J., Caley, W.F., Stewart, i. and **Whiteway, S.G.** 1984. "Decomposition of Pyrite and Trapping of Sulphur in a Coal Matrix During Pyrolysis of Coal," *Fuel 63:* 1579-1582.
Fine, H.A., Lowry, M., Power, L.F., and **Geiger, G.H.** 1976. "A Proposed Process for the Desulfurization of Finely Divided Coal by Flash Roasting and Magnetic Separation." *IEEE Trans. on Magnetics MAG-12:* 523-527.
Hirajima, T., Chan, E. and **Whiteway, S.G.** 1986. "Vacuum and Atmospheric Pressure TGA on an Eastern Canadian Coal." *Fuel 65:* 844-848.
Hirajima, T., Chan, E., Whiteway, S.G., Stefanski, M. and **Stewart, I.** 1987. "Beneficiation of Char, from a Pyrolysed Eastern Canadian Coal, by Aggregative Flotation." *Coal Preparation* 5: 85-108.
Jagadeesh, M.S. and **Seehra, M.S.** "Thermomagnetic Studies of Conversion of Pyrite and Marcasite in Different Atmospheres." J. *Phys. D. Appl. Phys. 14:* 2153.
McCready, R.G.L. 1984. *"Microbiological Studies on High-Sulphur Coals,"* Atlantic Research Laboratory Technical Report No. 50.
Mikhlin, J. 1987. *"Beneficiation Potential of Eastern Canadian Coals,"* Final report under Dept. Supply and Services Contract O.S.C. 85-00494.
Moore, R.S., 1982. *"Beneficiation Techniques Applicable to Nova Scotian Coals: A Literature Survey."* Atlantic Research Laboratory Technical Report No. 39.
Roy, C. and **DeCaumia, B.** 1984. *"Vacuum Pyrolysis of Prince Mine Coal,"* Atlantic Research Laboratory Technical Report No. 46.
Stewart, I. Whiteway, S.G., Cleyle, P.J. and **Caley, W.F.** 1986. *"Decomposition of Pyrite in a Coal Matrix during the Pyrolysis of Coal."* ACS Symposium Series No. 301 — Mineral Matter and Ash in Coal. Karl S. Vorres, Editor.
Stirling, J.A.R. 1984. *"Influence of the Mineralogy of Coal, and in Particular of Sulphur Compounds, on the Possibility of Sulphur Removal and Ash Reduction,"* Atlantic Research Laboratory Technical Report No. 48.
Whiteway, S.G. and **Galvez, I.** 1978. "The Desulphurization of Coal During Coking by the Injection of Sulphur-Free Coke Oven Gas," *Proc. Chem. Eng. Conference, Chemical Institute of Canada: 78-87.*

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A COMPARISON OF FUNGAL FLORAS OF HIGHLAND AND LOWLAND PASTURE IN ICELAND[1]

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Fifty-four soil samples were collected during the summers of 1982 nd 1983 from a reclaimed bogland (elevation 20 m = lowland) pasture at approximately 64°N, 21°W and 40 soil samples were obtained in the same time period from a highland (elevation 474 m = highland) pasture at about 64°N, 19°W. The mean number of fungal propagules per gram of soil of the former was 5.3×10^5 and of the latter 2.9×10^5 and the difference was significant at the $P < 0.001$ level. The species present in the two floras were different. *Gliocladium catenulatum, Paecilomyces carneus* and *Pseudeurotium zonatum* were present in more than 60% of the lowland soil samples and accounted for 21% of the total flora, but were not found in the highland pasture soil. Conversely, the most common species found in the highland soil *Penicillium* sp. 525 was not isolated from the lowland soil samples.

Cinquante-quatre (54) échantillons de sol ont été récoltés d'un pâturage réclamé d'un terrain marécageux (élévation 20 m = bas terrain) situé à 64°N, 21°W au cours des étés de 1982 et 1983. Quarante échantillons (40) de sol ont été obtenus d'un pâturage élevé (élévation 474 m = haut terrain) situé à 65°N, 19°W pendant la même période. La quantité moyenne de propagules fongiques de sol provenant du haut et du bas terrain était respectivement de 5.3×10^5 et 2.9×10^5 par gramme de sol. La différence est significative au niveau de $P < 0.001$. Les espèces présentes dans les deux flores étaient différentes. *Gliocladium catenulatum, Paecilomyces carneus* et *Pseudeurotium zonatum* étaient présentes dans plus de 60% des échantillons provenant du bas terrain et constituaient 21% du total des flores, mais elles n'ont pas été trouvées dans les échantillons provenant du haut terrain. Inversement, l'espèce la plus commune dans les échantillons provenant du haut terrain, *Penicillium* sp. 525, n'était pas présente dans les échantillons provenant du bas terrain.

Introduction

It has been shown that the daily weight gain of lambs on highland pastures in Iceland is about 270 g while the gain of similar lambs on reclaimed bogland in southern Iceland is about 230 g and that this difference is statistically highly significant and extends to the weight and quality of the carcasses (Gudmundsson, 1987). The reasons for this difference are unknown.

Recently evidence has accummulated which indicates that metabolites of several fungi from the soils of permanent pasture affect the metabolism of bacteria that are functionally important in digestion of feed in the rumen (Brewer *et al.*, 1979; Jen and Jones, 1983; Liss *et al.*, 1985). Thus it appeared that the ill-thrift phenomenon in Iceland might be due in part to such an interaction. We have therefore examined the fungal flora of soil which supported herbage where ill-thrift was common and compared it with the flora of soil of pasture where good performance was achieved. The results of these studies are reported.

Materials and Methods

Collection of soil samples. - The geographical location of the experimental plots is shown on the maps (Fig 1A and Fig 1B). Each plot was divided into 10 sectors as shown in Fig 2A for the lowland plot and Fig 2B for the highland. Samples were collected from 5 sectors of each experimental plot as indicated in Tables I and III, except for the

[1] NRCC No. 29556

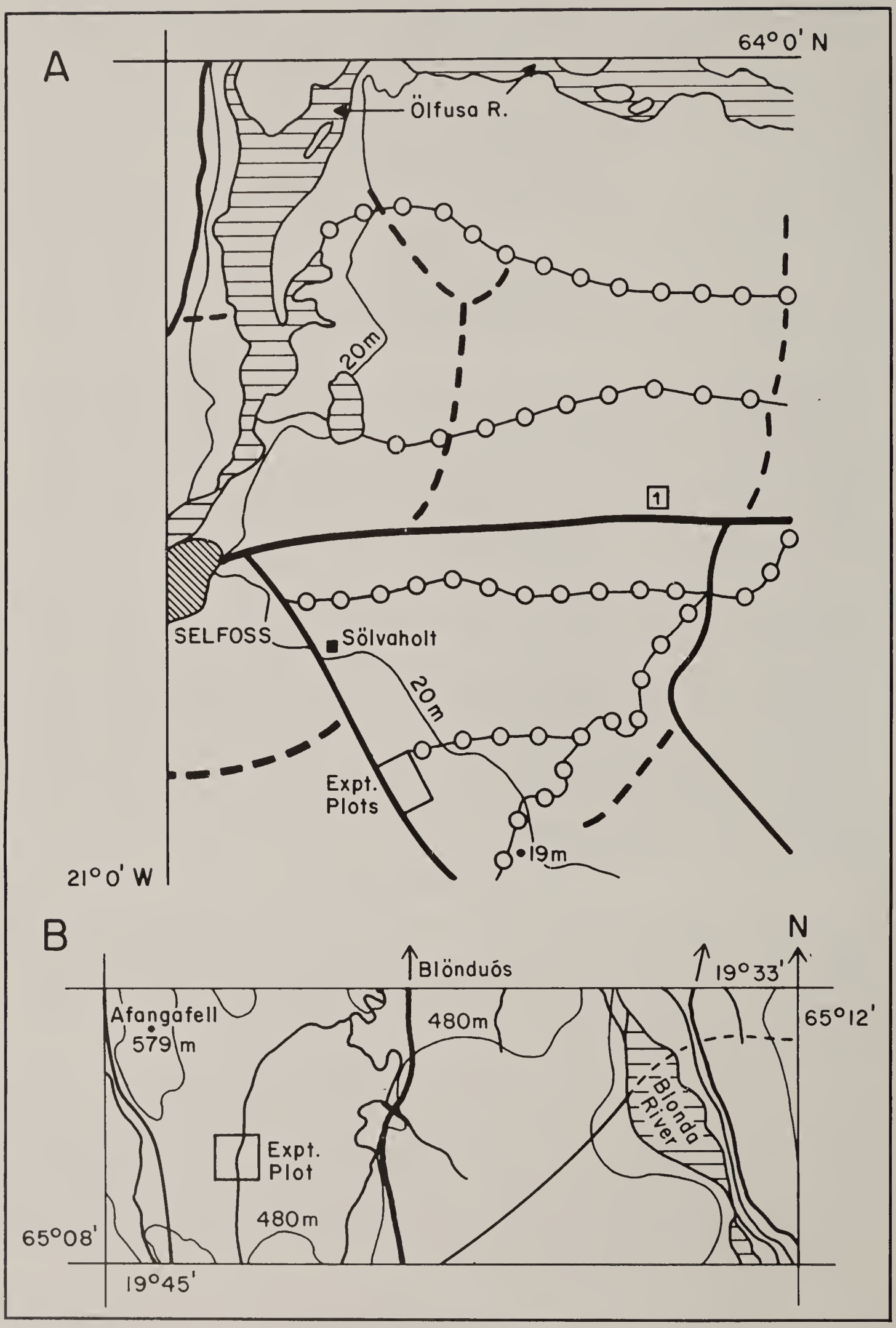

Fig 1 A. Map (1:100,000) of location of lowland plot; —— 20 m contour, —○— drainage ditches, ≡ river, [——] roads, [– – –] track; B. Map (1:100,000) of location of highland plot. The maps are based on the Iceland 1:100,000 series with much detail omitted from the drawings.

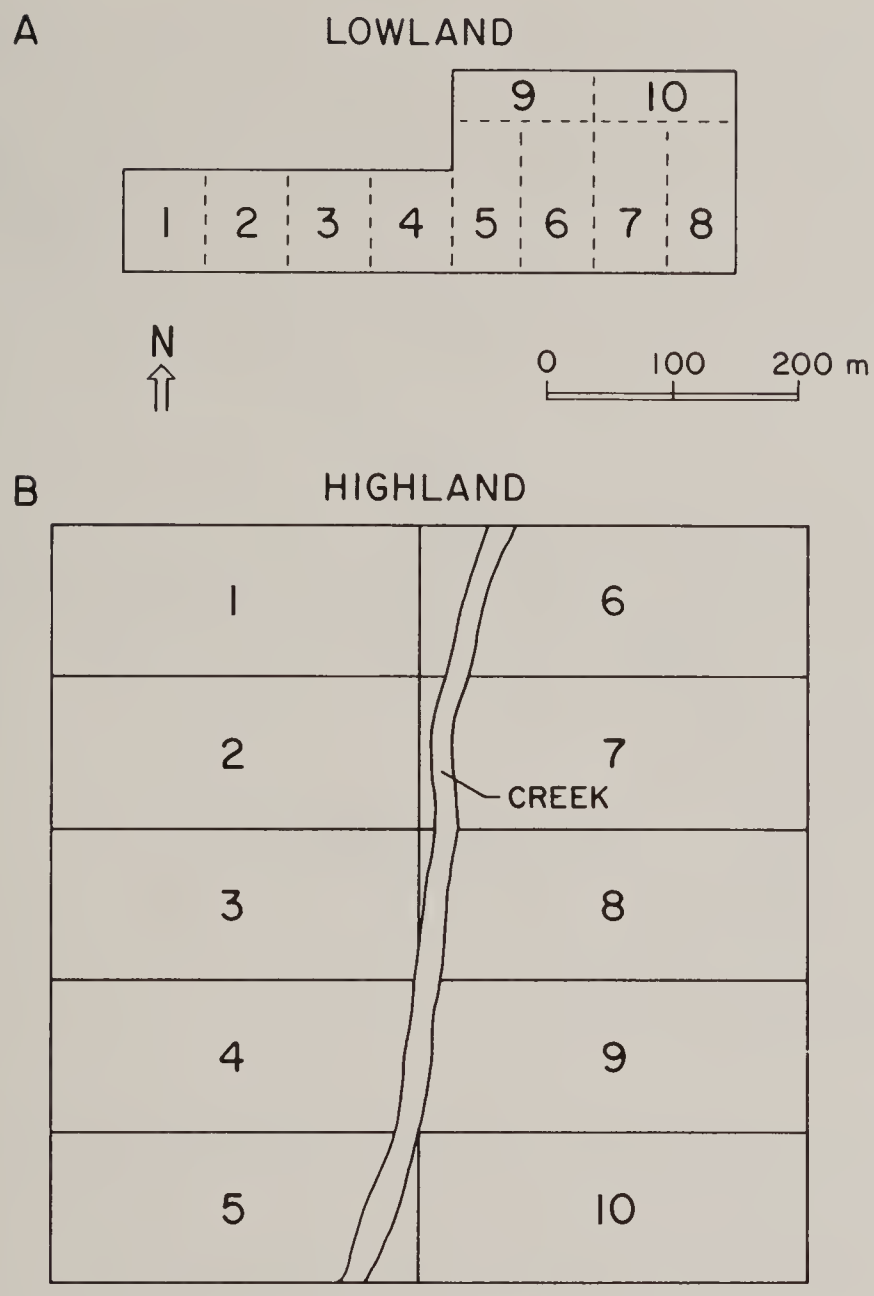

Fig 2 A. Location of sectors on lowland plot; B. Location of sectors on highland plot.

initial sampling of the lowland plot, where only 4 samples were collected. The sectors from which soil cores were obtained on particular dates were randomly selected and the method of collection was identical to that described by Brewer et *al.*, 1971. At the time of collection, the soil temperatures at the 1 and 5 cm depths were recorded. The rainfall occurring in the 3 weeks prior to the first sampling date of each year and thereafter the rainfall between sampling dates was recorded.

Collection of fungi from the herbage of experimental plots. - Fungal spores were collected from the herbage by means of a spore trap. A drawing of the trap is given in Fig 3. The trap was operated by sucking air through the filters whilst walking on the pasture. The trap was swung laterally during collection so that the rubber flaps disturbed the herbage. It was calibrated by calculating the volume of air sucked through the filters by one stoke of the pump. Each filter from the trap was washed with distilled water (50 mL), the spores dispersed by shaking and aliquots (1-5 mL) were filtered through filter discs of diameter 13 mm and porosity 1.2 μm (Millipore Corporation, Bedford, Mass.). The resulting filter discs were air-dried, mounted in 70% lactic acid on microscope slides and all the spores on each disc were counted. This number was converted to the number of spores found in each litre of air passed through the trap at standard temperature and pressure.

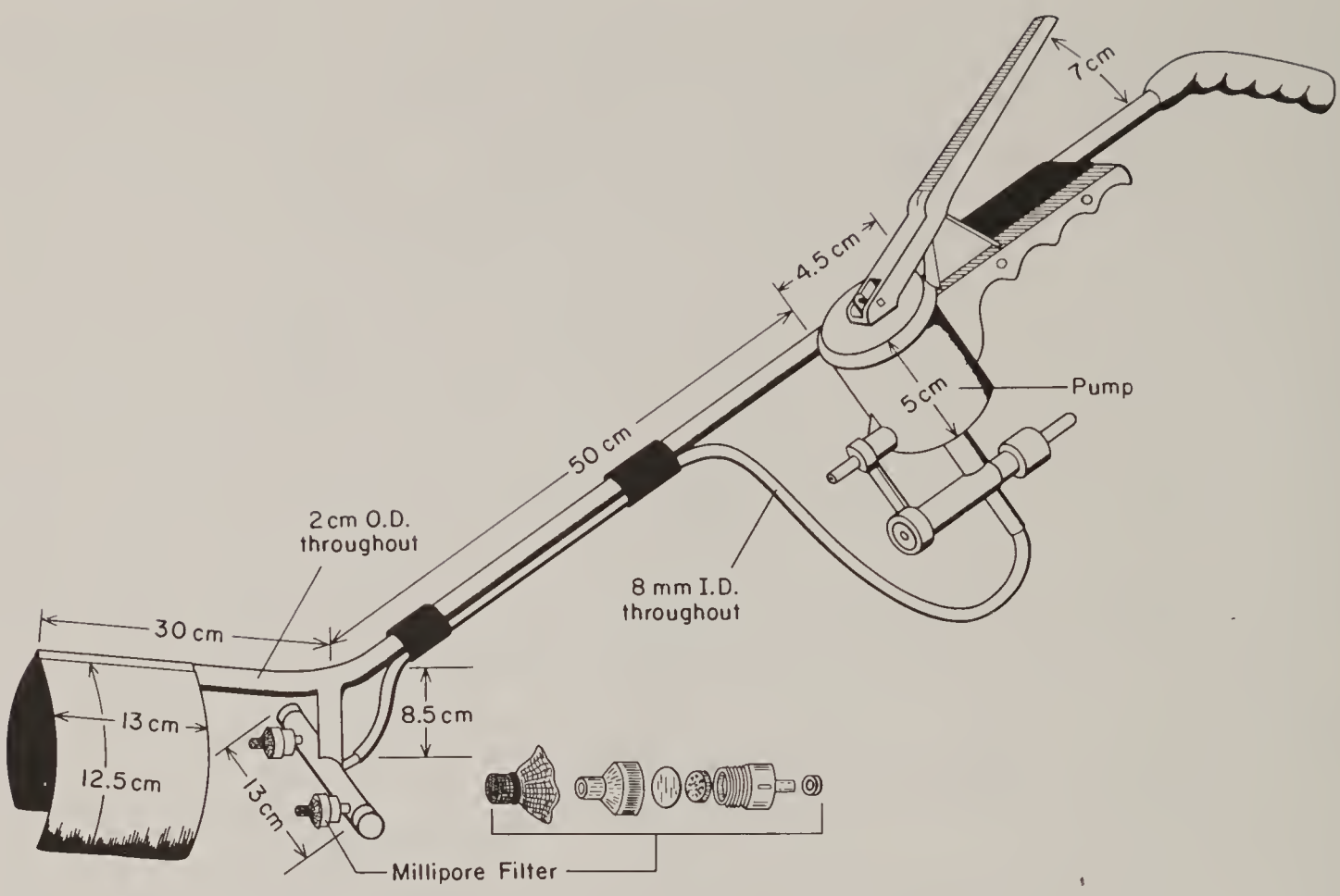

Fig 3 Diagram of spore trap.

Isolation of fungal propagules. - The time that elapsed between the collection of the samples and the dilution plating was 3 to 4 hours for the lowland and 15 to 20 hours for the highland samples.

The method of dilution plating was described by Brewer et al., 1971, with the addition to the medium of soluble starch (1 g L^{-1}) and cellulose (6 g L^{-1}, MN300, Nagel & Co). The plates were incubated for 8 to 12 days at 25°C and a subculture was made from each colony that appeared to be different from any other colonies. The number of colonies of each type that occurred on the 10 plates of the dilution from which the subculture was made (10^5 or 10^4) was recorded together with the ability to hydrolyze starch and cellulose, the ability to reduce rose bengal, and the growth-inhibiting effect (if any) on adjacent fungal colonies (Brewer and Taylor, 1980). After incubation of 1 to 2 weeks, the cultures were sent to the Atlantic Research Laboratory for identification.

Recording of field data, identity and physiological characteristics of fungal populations. - The field data in the previous paragraph and meteorological data were recorded in field books as previously described (Brewer and Taylor, 1980). This information together with the results of taxonomic studies and antibiotic screening were transferred to machine readable form as described (Brewer and Taylor, 1980). All computations were done on a Control Data Cyber 170-730 computer. Some of the programs used have been published (Brewer & Taylor, 1980). Statistical analyses of the data were performed using programs in the BMDP (Dixon, 1985) package.

Selection of random samples. - The data recorded in the field books was used to assemble a theoretical soil fungal population based on the frequency of morphologically distinct colonies on the isolation plates. Six different samples each of 150 isolates were selected at random from this theoretical population and the similarity of each random sample to the total population scored. This procedure applied to the floras of both the highland and lowland plots has been described in detail (Brewer and Taylor, 1980).

Growth inhibitory activity of the isolates in random samples. - All viable cultures in the random samples from the two plots were grown in petri dishes on the following medium: molasses 20 g, dextrin 30 g, fish meal 15 g, "Pharmamedia" (Traders Protein Division, Forth Worth, Texas) 15 g, agar 20 g and distilled water 1000 mL. The isolates were screened for antibiotic production according to the method of Brewer *et al.*, 1974. This assay employed *Micrococcus luteus* (HLX 701)* and *Candida utilis* (HLX 910), the media and temperatures of incubation of the assay organism were nutrient agar (Difco) at 35°C (*M. luteus*) and 2% malt agar at 25°C (*C. utilis*).

Results

The meteorological data in Fig 4 show that in both years of this experiment the two experimental plots experienced soil temperatures of about 10° and rainfall exceeding 20 mm month^{-1} (generally 50 mm month^{-1}) over the period June to September. These

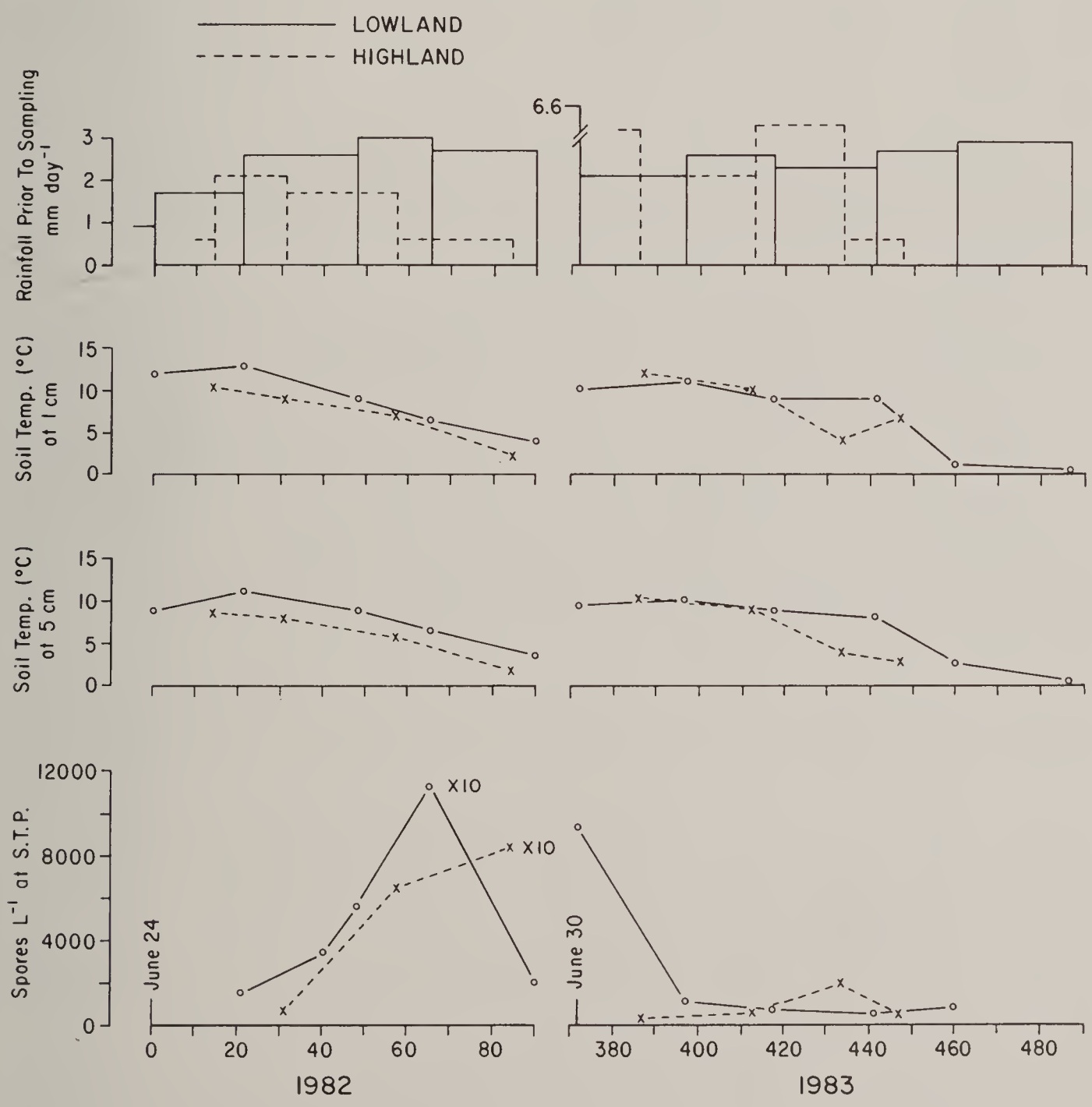

Fig 4 Soil temperatures, rainfall and spore numbers per litre of air on lowland and highland plots 1982 and 1983.

* Accession No. to culture collection at the Atlantic Research Laboratory.

Table I Dates of collection from the lowland plot indicating the sectors sampled and the calculated number of propagules per gram of soil (x 10^{-4}).

Day No.	Date	Sector Number 1	2	3	4
1	24/6/82				57
21	14/7/82	76	24 (H)		29
48	10/8/82	43			40 (H)
65	27/8/82	43		17	
90	21/9/82	31 (H)		36	
372	30/6/83		25	16 (H)	37
397	25/7/83		42		
418	15/8/83		48		162
442	8/9/83		30		
461	27/9/83		18	38 (H)	
488	24/10/83		43		115 (H)
Mean ± SD		48	33 ± 11	27	73 ± 53
No. of isolates in collection		116	201	94	161

benign conditions led inevitably to fungal growth and this is clearly demonstrated in Fig 4 where fungal efflorescences resulted in spore densities greater than 10^5 L^{-1} air on the lowland pasture and 8 x 10^4 on the highland.

Fungal populations of the soil of experimental plots

Lowland Plot. Fifty four soil samples were collected from this plot on 11 collection days. The dates of collection and the sectors of the plot from which they were acquired are indicated in Table I. The mean number of propagules collected in each gram of soil was 5.3 x 10^5 ± 3.5 x 10^5. The mean values for each sector are also given in Table I and although few samples were collected it is clear from the standard deviations that there were no differences among those sectors from which 6 or more samples were obtained. We thought that there might be differences in the fungal populations of the soil beneath the hummocks (samples in Table I marked (H)) in the experimental plots and the flora from that of the flat pasture. However, there appears

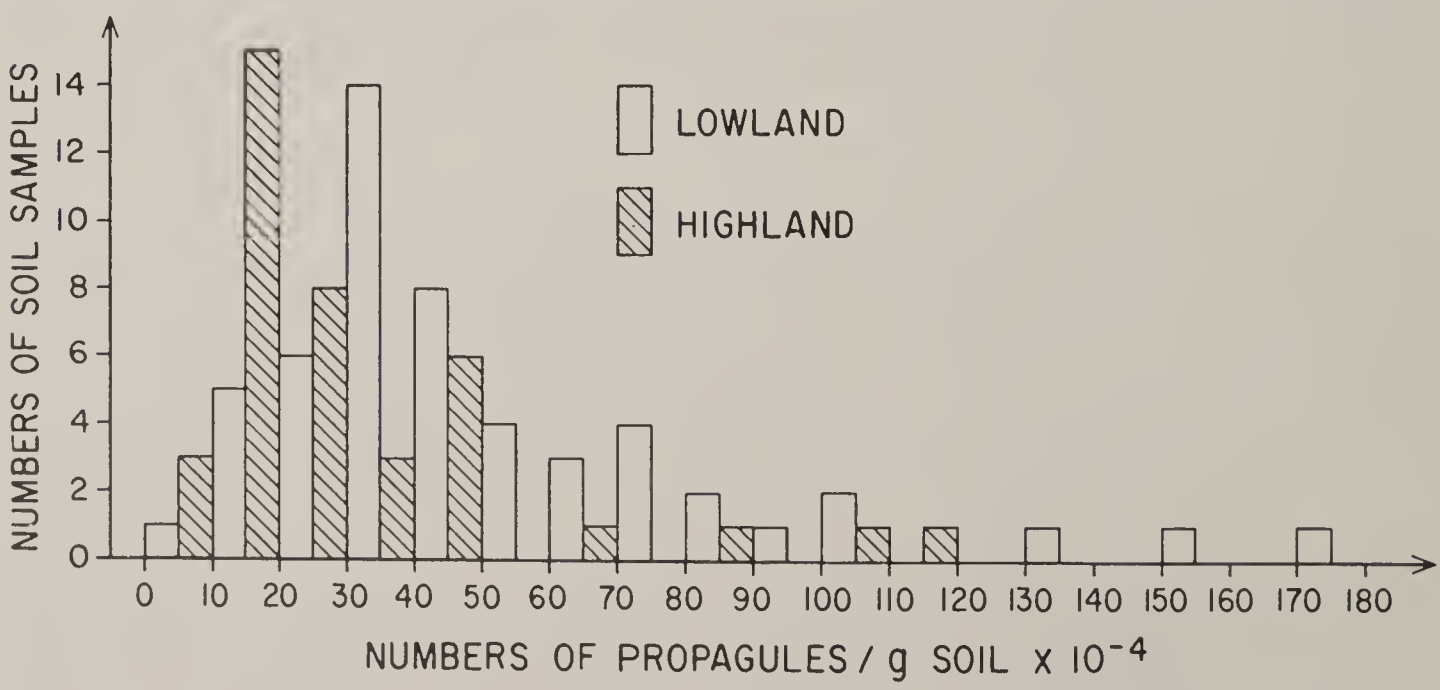

Fig 5 Histograms of numbers of fungal propagules per gram of dry soil collected in 1982 and 1983 on highland and lowland pastures.

Sector Number					
5	6	7	8	9	10
		26	37	9	
			19 (H)		102
	25	36 (H)			39
	76 (H)			15 (H)	46
		33 (H)	61 (H)		70 (H)
		57 (H)	50		
81 (H)		39	61 (H)	58	
33 (H)		75	44 (H)		
	88	29	106 (H)	39 (H)	
	32 (H)		64		38 (H)
135		90 (H)	172 (H)		
81	48	48 ± 24	68 ± 46	37	59
110	95	260	277	114	161

to be no difference in the two populations (mean number $5.5 \times 10^5 \pm 3.7 \times 10^5$, n = 24 under the hummocks and $5.1 \times 10^5 \pm 3.4 \times 10^5$, n = 30 on flat pasture). The data in Table I are also shown as a histogram in Fig 5 to illustrate the distribution of the population in groups of 10^5. When these results were subjected to a linear least mean squares test the gradient of the fitted line was 0.06 and it may be concluded that a change in population numbers during the experimental period did not occur.

The number of fungal isolates cultivated from these fifty-four soil samples was 1589 and their mean frequency was 1.8×10^5. Twenty-eight percent of these isolates have been classified with respect to species, or genus or have been assigned a taxonomic number on the basis of their characteristic morphology. A list of these fungi is given in Table II together with their frequencies, the number of soil samples in which they occurred, and the temperature range recorded for the soil sample at a depth of 1 cm when it was collected. Of these 50 species only 10 were found in 10 or more soil samples. In all cases they were isolated form soil plugs over the whole temperature range (0°-15°) and the sum of their frequencies (1.18×10^7) account for 41% of all the propagules collected (2.86×10^7). Further, four of these species *Gliocladium catenulatum, Paecilomyces carneus, Pseudeurotium zonatum and a Cylindrocarpon* sp. were found in almost 60% of the soil samples and the sum of their frequencies account for 23% of all the propagules collected.

Highland Plot. Forty soil samples were collected from this plot on 8 collection days. The dates of collections and the sectors of the plot from which they were obtained are indicated in Table III. The mean number of propagules collected in each gram of soil was $3.6 \times 10^5 \pm 5.5 \times 10^5$. Comparison of this result with that from the lowland plot using Student's "t" test showed that the variances of the two populations were the same (F value 0.79) and that the difference of the means was significant ($P < 0.1$). The number of propagules collected from sector 6 on 14/9/83 (Table III) was about 3 times greater than that of the sample (sector 8, 31/8/83) with the next highest population density on this plot and almost twice as high as that isolated from any other soil sample (lowland plot, sector 8, 14/10/83). If this high result is omitted from the analysis the mean value of propagules isolated from the highland plot becomes $2.9 \times 10^5 \pm 2.5 \times 10^5$ and the difference between the highland and the lowland plots becomes significant at the $P < .001$ level. The mean values for each sector are given in Table III and apart from the anomalous sector 6 it is clear that there were no significant differences.

Table II List of classified fungi from soil of the lowland plot, numbers of propagules per gram of soil, their ability to hydrolyse starch, reduce rose bengal and inhibit the growth of other fungi.

Species		Propagules $\bar{M}$ [a]	Number of Soil Samples	Isolates in Collection	Temp. Range °C	Activity Antifung. $\bar{M}$ [a]	n	Starch hydrol. $\bar{M}$ [a]	n	Rose Bengal reduction $\bar{M}$ [a]	n	Known Metabolite(s)
Acremonium butyri (van Beyma) Gams		1	1	1	10-12	0	0	0	0	0	0	-
Alternaria alternata (Fr.) Keissler		22	3	4	4-15	0	0	0	0	0	0	AK-toxins
Apiospora montagnei Sacc.		12	2	2	10-12	0	0	0	0	12	2	-
Aspergillus repens De Bary		1	1	1	13-15	0	0	0	0	0	0	-
Aspergillus sp.	(110)	10	1	1	13-15	0	0	10	1	0	0	
Botrytis cinerea Pers. ex Nocca & Balb		32	2	4	10-15	0	0	0	0	11	2	Botrylacton
Cephalosporiopsis sp.	(794)	5	3	3	7-15	1	1	0	0	0	0	
Chaetomium cochliodes Palliser		9	2	2	4-6	0	0	0	0	0	0	Cochliodinol
Chaetomium funicolum Cooke		1	1	1	10-12	0	0	0	0	0	0	-
Chaetomium sp.	(200)	10	1	1	13-15	0	0	0	0	0	0	
Cladosporium cladosporioides (Fresen.) de Vries		7	1	1	13-15	0	0	0	0	0	0	Cladosporolides
Cladosporium herbarum (Pers.) Link ex S.F. Gray		10	3	3	13-15	0	0	7	1	0	0	Mycosporins
Cladosporium sphaerospermum Penz.		20	1	1	13-15	0	0	0	0	0	0	+
Cylindrocarpon sp.	(495)	21	32	46	0-15	0	0	11	2	18	15	
Epicoccum nigrum Link ex Link		1	1	1	13-15	0	0	0	0	0	0	Epicorazines
Fusarium sp.	(270)	0.8	1	1	4-6	0	0	0	0	0	0	

Gliocladium catenulatum Gilm. & Abbott	94	36	67	0-15	19	1	152	2	45	5	-
Humicola fuscoatra Traaen var. *fuscoatra*	9	1	1	7-9	0	0	0	0	0	0	-
Mortierella minutissima van Teigh.	20	1	1	10-12	0	0	0	0	0	0	-
Mucor hiemalis Weimar	6	20	22	0-15	0	0	0	0	10	2	-
Oidiodendron griseum Robak	6	1	1	10-12	0	0	0	0	0	0	-
Oidiodendron tenuissimum (Peck) Hughes	12	2	2	0-9	0	0	0	0	0	0	-
Paecilomyces carneus (Duché & Mein) Brown & Smith	36	35	48	0-15	10	1	16	4	37	27	Gliotoxin
Paecilomyces sp. (180)	8	1	1	4-6	0	0	0	0	0	0	
Paecilomyces sp. (1810)	22	2	3	10-12	0	0	0	0	0	0	
Penicillium waksmanii Zaleski	1	1	1	10-12	0	0	1	1	1	1	-
Pseudeurotium zonatum van Beyma	37	33	40	0-15	2	1	0	0	20	2	Cytochalasin G
Thielavia sp. (1512)	40	27	34	0-15	41	2	77	1	38	6	
Tolypocladium cylindrosporum Gams	23	2	2	4-6	0	0	0	0	0	0	-
Trichocladium opacum (Corda) Hughes	26	25	34	0-15	0	0	18	6	10	1	-
Trichocladium sp. (4181)	22	5	6	0-12	0	0	29	1	38	1	
Trichoderma hamatum (Bon.) Bain.	15	6	8	0-15	38	1	0	0	10	2	Dermadin
Trichoderma harzianum Rifai	2	2	3	13-15	0	0	0	0	0	0	Isocyanides
Trichoderma viride Pers. ex Gray	9	10	12	0-15	0	0	0	0	9	6	Viridin
Trichoderma sp. (170)	19	17	20	0-15	0	0	0	0	21	13	
Truncatella angustata (Pers. ex Lk.) Hughes	5	6	6	4-12	0	0	1	1	0	0	-

Table II List of classified fungi from soil of the lowland plot, numbers of propagules per gram of soil, their ability to hydrolyse starch, reduce rose bengal and inhibit the growth of other fungi (continued).

Species		Propagules $\overline{M}$ [a]	Number of Soil Samples	Isolates in Collection	Temp. Range °C	Activity: Antifung. $\overline{M}$ [a]	Antifung. n	Starch hydrol. $\overline{M}$ [a]	Starch hydrol. n	Rose Bengal reduction $\overline{M}$ [a]	Rose Bengal reduction n	Known Metabolite(s)
Ulocladium atrum Preuss		1	1	1	10-12	0	0	0	0	0	0	-
Ulocladium consortiale (Thüm) Simmons		37	6	8	4-15	0	0	64	2	70	2	-
Unknown	(781)	36	15	16	0-15	49	2	59	1	34	11	
Unknown	(782)	12	1	1	0-3	0	0	0	0	12	1	
Unknown	(783)	157	4	4	4-15	0	0	580	1	0	0	
Unknown	(784)	2	1	1	10-12	0	0	0	0	0	0	
Unknown	(785)	6	2	2	10-12	0	0	0	0	11	1	
Unknown	(787)	2	1	1	13-15	0	0	2	1	0	0	
Unknown	(788)	22	1	1	13-15	0	0	22	1	0	0	
Unknown	(789)	10	4	4	7-12	0	0	0	0	0	0	
Unknown	(790)	157	7	7	0-12	0	0	0	0	181	6	
Unknown	(791)	114	7	8	0-12	0	0	0	0	135	4	
Verticillium cephalosporum Gams		23	3	3	0-12	0	0	0	0	0	0	-
Verticillium lecanii (Zimm.) Viégas		36	2	3	0-15	0	0	0	0	0	0	Bassianolide

[a] $\overline{M}$ = mean number of propagules per gram of soil x 10^{-3}. n = number of soil samples in which the species were found. A dash in column 13 indicates that this species has no known toxic metabolites. "+" indicates that this species has been reported to produce toxic metabolites which were not characterized.

Table III Dates of collection from highland plot indicating the sectors sampled and the calculated number of propagules per gram of soil (x 10^{-4}).

Day No.	Date	Sector Number 1	2	3	4	5	6	7	8	9	10
14	7/7/82	1		19	17 (H)			23		16	
31	24/7/82			23		11 (H)		12 (H)	19	18	
57	19/8/82		30 (H)	21		20		15 (H)			107 (H)
84	15/9/82	46		31	6 (H)		28 (H)	24 (H)			
387	15/7/83	11 (H)		40	21	11				84 (H)	
413	10/8/83		18 (H)	26	1			60 (H)	41		
434	31/8/83		15		49				116	40 (H)	43 (H)
448	14/9/83		10			14 (H)	342 (H)	16 (H)			32 (H)
Mean ± SD		19	18	27 ± 8	19	14	185	25 ± 18	59	40	61
No. of isolates in collection		84	127	177	110	102	54	151	73	110	84

Table IV List of classified fungi from the soil of the highland plot, numbers of propagules per gram of soil, their ability to hydrolyse starch, reduce rose bengal and inhibit the growth of other fungi.

Species	Propagules M	Number of Soil Samples	Isolates in Collection	Temp. Range °C	Activity Antifung. M	Antifung. n	Starch hydrol. M	Starch hydrol. n	Rose Bengal reduction M	Rose Bengal reduction n	Known Metabolite(s)
Alternaria alternata	7	5	5	4-12	0	0	9	2	0	0	Tenuazonic acid
Botrytis cinerea	5	3	3	7-12	0	0	0	0	6	1	Botrydiol
Chaetomium cochliodes	85	1	1	7-9	0	0	0	0	0	0	Chetomin
Cladosporium cladosporioides	3	1	1	10-12	0	0	0	0	0	0	Asperentin
Cladosporium herbarum	7	3	4	10-15	0	0	7	1	0	0	Mycosporins
Cladosporium sphaerospermum	7	2	3	7-15	0	0	1	1	0	0	+
Cylindrocarpon sp. (495)	4	1	2	16-18	0	0	0	0	0	0	
Fusarium sp. (270)	0.8	1	1	16-18	0	0	0	0	0	0	
Humicola fuscoatra var. *fuscoatra*	1	1	1	7-9	0	0	0	0	0	0	-
Humicola grisea Traaen	3	1	1	7-9	0	0	0	0	0	0	-
Mortierella ramanniana (Möller) Linnem.	13	1	1	10-12	0	0	0	0	0	0	-
Mortierella vinacea Dixon-Stewart	42	7	9	4-12	0	0	0	0	63	4	-
Mucor hiemalis	0.5	1	2	10-12	0	0	0	0	0	0	-
Oidiodendron truncatum Barron	7	1	1	10-12	0	0	0	0	0	0	PR 1350
Paecilomyces sp. (1809)	13	1	1	10-12	0	0	13	1	0	0	
Penicillium sp. (525)	46	8	11	0-15	0	0	57	5	100	1	
Penicillium sp. (526)	14	3	4	13-15	0	0	14	3	0	0	
Penicillium sp. (527)	18	1	1	7-9	0	0	0	0	0	0	
Penicillium notatum Westling	9	1	1	7-9	0	0	0	0	0	0	Penicillin
Tolypocladium cylindrosporum	10	2	4	4-15	0	0	0	0	0	0	-

Tolypocladium niveum (Rostrup) Bisset		3	5	5	4-12	0	0	0	0	0	0	-
Trichocladium opacum		10	19	23	4-18	0	0	13	2	0	0	-
Trichoderma polysporum (Link ex Pers.) Rifai		0.2	1	1	10-12	0	0	0	0	0	0	Cyclosporins
Truncatella angustata		5	2	2	7-12	0	0	0	0	0	0	-
Ulocladium consortiale		23	5	6	0-12	0	0	0	0	6	1	-
Unknown	*(785)*	35	1	1	10-12	0	0	0	0	35	1	
Unknown	*(786)*	7	1	1	10-12	0	0	0	0	0	0	
Unknown	*(793)*	6	1	1	10-12	0	0	0	0	0	0	
Verticillium lecanii		3	3	3	7-12	0	0	0	0	0	0	Bassianolide
Verticillium sp.	(9351)	2	1	1	7-9	0	0	0	0	0	0	
Zygorrhynchus moelleri Vuill.		6	4	4	4-15	0	0	0	0	0	0	-

Abbreviations are explained in Table II.

Table V Biological activities of numbers of unclassified isolates from soils of highland and lowland pastures

Biological Activity	Lowland		Upland	
	Total Collection	Random Sample	Total Collection	Random Sample
Starch Hydrolysis	58	6	96	11
Rose Bengal Reduction	205	31	162	26
Growth Inhibition of other soil fungi	16	4	24	8
Growth inhibition of *M. luteus*		22		7
Growth inhibition of *C. utilis*		21		10

Table VI Days of collections appearing in the random sample from the lowland pasture indicating the sectors sampled and the calculated number of fungal propagules per gram of soil ($\times 10^{-4}$).

	Sector Number									
Day No.	1	2	3	4	5	6	7	8	9	10
1				15			8	1		
21	31	4		3						37
48	12			6			4			9
65	10		4							1
90	8		13				3	19		12
372		4	2				22	1		
397		12			6		12	11	26	
418		3		6	7		21	1		
442		2				10	13	53	3	
461			2			3		28		10
488		27		78	5		42	109		
Mean ± SD	15	13 ± 10	5	22	6	6	16 ± 13	27 ± 37	14	14
No. of isolates selected	11	13	8	18	5	7	27	25	8	17
% of isolates in collection	9	6	9	11	5	7	10	9	7	11

Some of the data in Table III are shown in Fig 5 where the population is sorted into groups of 10^5 illustrating the differences between the lowland and highland populations. The results from the highland plot were subjected to a linear least mean squares regression analysis and the gradient of the line obtained, 0.07, indicates the numerical stability of the population during the experimental period.

The number of fungal isolates cultivated from these 40 soil samples was 1072 and their mean frequency was 1.4×10^5. One hundred and eleven (10%) of these isolates have been classified, as defined for the lowland plot. A list of these fungi is given in Table IV, together with their frequencies, the number of soil samples in which they were found and the temperature range recorded for the soil samples at a depth of 1 cm when it was collected. Only 6 of these 31 species were found in 5 or more of the soil samples and these 6 accounted for 9% of the total propagules collected (1.18×10^6). None of the isolates inhibited the growth of other fungi on the isolation plates and only one organism (*Penicillium* sp. 525) was active in more than one test; 11 out of 31 were demonstrated to have biological activity (Table IV). This contrasts to the biological activity of the isolates from the lowland plot, where 27 out of 50 showed some activity and 20% had activity in two or more tests (Table II). The physiological properties of the unclassified isolates are summarised in Table V. The greater ability of the highland population to hydrolyse starch (115 from highland pasture vs 89 from the lowland) was mostly a property of the unclassified fungi (highland 96, lowland 58) and all the organisms that inhibited the growth of other fungi on the isolation plates from the highland soil samples remain unclassified.

Selection of a random sample of 150 isolates from the fungi of the soil of highland and lowland pastures. - A theoretical flora of 21044 organisms was assembled from isolates from the lowland plot and 6 random selections of 150 isolates were made from this flora. The selections were scored as described (Brewer and Taylor, 1980) and the one having the highest score had the following characteristics (characters of the whole population given in parentheses): number of collection dates, 11 (11); mean frequency (isolates g^{-1}) 1.9×10^5 (1.8×10^5); gradient of linear least mean squares plot 0.016 (0.06); number of soil samples 46 (54): number of different isolates 139: geographical distribution - standard deviation of percent isolates selected for each sector 2.06; classified number of organisms 18 (50). Similarly the collection from the highland plot was expanded to 10235, the selections scored in the same way, the one

Table VII Days of collections appearing in the random sample from the highland pasture indicating the sectors sampled and the calculated number of fungal propagules per gram of soil ($\times 10^{-4}$).

	Sector Number									
Day No.	1	2	3	4	5	6	7	8	9	10
14	1		4	1			11		1	
31			7		2		3	6	10	
57		12	4		8		7			35
84	21		10			8	2			
387	1		7	8	1				17	
413		3	3				28	17		
434		1		37				69		18
448					5	93	3			3
Mean ± SD	7	5	6	15	4	8	9±10	31	9	19
No. of isolates selected	8	8	20	12	13	9	18	15	17	12
% of isolates in collection	10	6	11	11	13	17	12	21	13	14

Table VIII List of classified fungi of the random sample from the lowland collection and their biological activity.

Species	Propagules M	Number of Soil Samples	Temp. Range °C	Activity of Cultures: Enzyme: Starch Hydrol. M	n	Enzyme: Rose Bengal reduction M	n	Growth Inhibition: *M. luteus* M	n	*C. utilis* M	n
Alternaria alternata	21	1	13-15	0	0	0	0	21	1	0	0
Cephalosporiopsis sp. (794)	13	1	7-9	0	0	0	0	0	0	0	0
Cylindrocarpon sp. (495)	16	3	10-15	21	1	19	2	15	2	10	1
Gliocladium catenulatum	68	8	0-15	100	1	49	2	68	6	0	0
Mucor hiemalis	13	2	7-15	0	0	14	1	0	0	0	0
Oidiodendron tenuissimum	230	1	7-9	0	0	0	0	0	0	0	0
Paecilomyces carneus	46	9	0-15	39	1	63	6	0	0	0	0
Paecilomyces sp. (1810)	22	1	10-12	0	0	0	0	22	1	0	0
Pseudeurotium zonatum	70	8	0-15	0	0	38	1	104	2	38	1
Thielavia sp. (1512)	14	3	0-15	0	0	0	0	0	0	0	0
Trichocladium opacum	83	4	0-15	0	0	0	0	38	2	36	1
Trichocladium sp. (4181)	10	1	4-6	0	0	0	0	10	1	0	0
Ulocladium consortiale	11	1	7-9	0	0	0	0	0	0	0	0
Unknown (781)	140	1	0-3	0	0	140	1	0	0	0	0
Unknown (783)	35	1	13-15	0	0	0	0	0	0	0	0
Unknown (790)	420	1	0-3	0	0	420	1	0	0	0	0
Unknown (791)	91	5	0-12	0	0	143	3	0	0	0	0
Verticillium cephalosporum	48	1	0-3	0	0	0	0	0	0	0	0

Abbreviations are explained in Table II.

Table IX List of classified fungi of the random sample from the highland collection and their biological activity.

Species	Propagules M	Number of Soil Samples	Temp. Range °C	Enzyme: Starch Hydrol. M	n	Enzyme: Rose Bengal reduction M	n	Biological Activity of Cultures: Growth Inhibition *M. luteus* M	n	*C. utilis* M	n
Chaetomium cochliodes	85	1	7-9	0	0	0	0	0	0	0	0
Mortierella vinacea	57	4	4-12	0	0	66	3	0	0	0	0
Paecilomyces sp. (1809)	13	1	10-12	13	1	0	0	13	1	0	0
Penicillium sp. (525)	55	4	0-15	55	4	100	1	100	1	0	0
Trichocladium opacum	20	5	7-12	18	1	0	0	18	1	7	1
Truncatella angustata	9	1	7-9	0	0	0	0	9	1	0	0
Ulocladium consortiale	33	3	0-9	0	0	0	0	43	1	0	0
Unknown (785)	35	1	10-12	0	0	35	1	0	0	0	0
Verticillium lecanii	9	1	7-9	0	0	0	0	0	0	9	1

Abbreviations are explained in Table II.

chosen having the characters: number of collection dates 8 (8); mean frequency (isolates g^{-1}) 1.4 x 10^5 (1.4 x 10^5); gradient of fitted line numbers vs time 0.04 (0.07); number of soil samples, 34 (40); number of different isolates 132; geographical distribution - standard deviation, 4.06; classified number of organisms, 9 (31). The distribution of soil samples selected is given in Table VI for the lowland plot and in Table VII for the highland plot. Comparisons of the biological activities of unclassified isolates in the random samples with the total collections are given in Table V.

Biological activities of random samples of fungal isolates from highland and lowland pastures. - Of the 139 different isolates in the random sample from the lowland pasture 28 proved to be non-viable on subcultivation on the antibiotic screening medium. The remaining 111 isolates were tested for their ability to produce metabolites that inhibited growth of *M. luteus* and *C. utilis*. The results of these experiments are summarised in Table VIII for the classified fungi and in Table V for the unclassified. Thirty-seven isolates (33%) of the total random sample produced metabolites that inhibited the growth of *M. luteus* and 10 isolates (9%) the growth of *C. utilis*.

Of the 132 different isolates in the random sample selected from the highland plot, 24 proved to be non-viable on subcultivation and the remaining 108 were assayed against *M. luteus* and *C. utilis*. The results of these experiments are summarised in Table IX for the classified organisms in the random sample and in Table V for the unclassified fungi. Thirty isolates (28%) of the random sample produced metabolites that inhibited the growth of *M. luteus* and 10 isolates (9%) the growth of *C. utilis*.

Discussion

In earlier work at Nappan, Nova Scotia small differences were established in numerical, taxonomic and some physiological characters of fungal populations in the soils of experimental plots that were only a few hundred meters apart. Though these plots, which had been managed similarly for at least 50 years, differed only in that one had been reclaimed from tidal marshland, the possibility exists that the smallness of the differences reported were due to some extent to the choice of methods.

By contrast, the experimental plots used in the work reported in this paper were widely separated and consequently were different with respect to climate, soil type and vegetation cover. Hence, if methods chosen to investigate the soil floras were valid it would be expected that larger differences in the floras of the two plots in Iceland would emerge than were found at Nappan.

The number of fungi on the lowland plot was almost twice that on the highland as compared with a difference of about 30% at Nappan. Taxonomically, at Nappan 83% of the fungi that were classified were found on both plots but in Iceland only 34% of the classified fungi were common to highland and lowland areas. At Nappan (Brewer et al., 1972) there was little difference in antibiotic activity when the isolates producing antibiotics were expressed as a percentage of those tested whereas in Iceland 42% of the organisms from the lowland and 37% of those from the upland produced growth inhibiting metabolites. These data, expanded in the Tables, support the expectation that greater differences would emerge between plots in Iceland.

Some of the species of fungi named in Tables II and IV are known to produce toxic metabolities when cultivated in the laboratory. The name of such a known metabolite of a species is given in the Tables. Of course, some species are known to produce several metabolites and this is indicated either by giving a generic name or by giving the names of different metabolities where the species appears in both Tables. Full details are readily available by consultation of the public "Mycotox" file (Brewer *et al.*, 1978). Of the four fungi which are most prevalent on the lowland plot only two —*Paecilomyces carneus* and *Pseudeurotium zonatum* are known to produce antibiotics. The latter has been reported (Probst and Tamm, 1981) to produce cytochalasin G

the activity of none of these metabolites against rumen bacteria has been reported to our knowledge.

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References

Brewer, D., Calder, F.W., MacIntyre, T.M. and **Taylor, A.** 1971. Ovine ill-thrift in Nova Scotia. I. The possible regulation of the rumen flora in sheep by the fungal flora of permanent pasture. *J. Agric. Sci., 71:* 465-477.

Brewer, D., Feicht, A., Taylor, A., Keeping, J.W., Taha, A.A. and **Thaller, V.** 1982. Ovine ill-thrift in Nova Scotia. 9. Production of experimental quantities of isocyanide metabolites of *Trichoderma hamatum. Can. J. Microbiol. 28:* 1252-1260.

Brewer, D., Hanson, A.W., Shaw, I.M., Taylor, A. and **Jones, G.A.** 1979. A crystalline toxic metabolite of *Trichoderma* spp. isolated from soil. *Experientia 35:* 294-295.

Brewer, D. and **Taylor, A.** 1980. Ovine ill-thrift in Nova Scotia. 6. Quantitative description of the fungal flora of soils of permanent pasture. *Proc. N.S. Inst. Sci., 30:* 101-133.

Brewer, D. and **Taylor, A.** 1986. *The fungal flora of pasture soil — a parameter in the ill-thrift syndrome of grazing sheep.* in *Grazing Research at Northern Latitudes* (Ed. O. Gudmundsson). Plenum Press, New York and London. pp. 279-287.

Brewer, D., Taylor, A. and **Evans, A.** 1978. A mechanical search and retrieval for mycotoxins. *Proc. N. S. Inst. Sci., 28:* 163-169.

Brewer, D., Taylor, A. and **Hoehn, M.M.** 1972. Ovine ill-thrift in Nova Scotia. II. The production of antibiotics by fungi isolated from forest and marshland soil. *J. Agric. Sci., 78:* 257-264.

Brewer, D., Taylor, A. and **Hoehn, M.M.** 1974. The antibiotic activity of cultures from fungal spores collected by a spore trap on permanent pasture. *Can. J. Microbiol. 20:* 721-729.

Dixon, W.J. (Ed.). 1985. *BMDP Statistical Software*. University of California Press, Berkeley, California.

Gudmundsson, O. 1987. Ill-thrift of suckling lambs on lowland pastures in Iceland. I. General characteristics and animal performance. *J. agric. Res. Iceland,* in the press.

Jen, W.-C. and **Jones, G.A.** 1983. Effects of chetomin on growth and acidic fermentation products of rumen bacteria. *Can. J. Microbiol. 29,* 1399-1404.

Liss, S.N., Brewer, D., Taylor, A. and **Jones, G.A.** 1985. Antibiotic activity of an isocyanide metabolite of *Trichoderma hamatum* against rumen bacteria. *Can. J. Microbiol. 31, 767-772.*

Probst, A. and **Tamm, C.** 1981. 19-*O*-acetylchaetoglobosin B and 19-*O*-acetylchaetoglobosin D, two new metabolites of *Chaetomium globosum. Helv. Chim. Acta 64:* 2056-2064.

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LOBSTER LARVAL ABUNDANCES IN LOBSTER BAY, YARMOUTH CO., NOVA SCOTIA - 1983

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A program to develop methods of capturing newly settled Stage IV larvae included the need to identify areas with significant planktonic concentrations of Stage IV larvae in Lobster Bay, Yarmouth Co., N.S. During the study, 171 larvae were collected, including 12 at four selected inshore stations studied by Stasko and Gordon (1983) in 1977-1978. Eighty-nine percent of all the larvae were captured within the environs of Whitehead Island. Exploratory towing, despite the wide range of sites, was the least successful category of stations (five larvae in 19 tows). A day-night towing cycle yielded the largest number of larvae at twilight. At Stasko's and Gordon's (1983) inshore stations, Stages I and IV exclusively were captured. Stage IV lobster larvae were rarely captured during our sampling, despite predominance of daytime towing. This survey differed from previous southwestern Nova Scotia surveys by sampling over shallow waters (less than 20 m) and adjacent to land.

Un programme pour développer des méthodes de capture pour les homards du stade IV qui commencent leur vie benthique incluait le besoin d'identifier des zones de haute concentration de larves de homard du stade IV dans Lobster Bay, comté Yarmouth, N.-É. Durant l'étude, 171 larves ont été capturées incluant 12 larves à 4 sites côtiers qui furent étudiées par Stasko et Gordon (1983) en 1977-1978. Quatre-vingt-neuf pourcent de toutes les larves ont été capturées dans les environs de l'Ile Whitehead. La catégorie des stations de trait d'exploration a été la moins fructueuse (cinq larves en 19 traits), malgré une grande variété de sites. Un cycle d'échantillonnage jour-nuit a produit le plus grand nombre de larves au crépuscule. Aux stations côtières de Stasko et Gordon (1983) des stades I et IV exclusivement ont été capturés. Les larves de homard du stade IV ont rarement été capturées malgré la prédominance d'échantillonnage durant le jour. Cette étude diffère des études précédentes au Sud-Ouest de la Nouvelle-Écosse car l'échantillonnage a été fait en eau peu profonde (moins de 20 m) et près des côtes.

Introduction

In 1971, the near-shore (to 92 km) lobster (*Homarus americanus* Milne Edwards) fishery of southwestern Nova Scotia (SWNS) (Fig 1) was expanded to a new offshore zone (>92 km from shore) (Pezzack and Duggan 1983). A lack of understanding of the relationship between offshore lobster stock and inshore stock emphasized the need for research on the early planktonic life stages of the lobster, and sources of recruitment in SWNS (Stasko 1978; Pezzack and Pringle 1986). Although considerable information is available on early developmental stages of *H. americanus* in the laboratory, distributional studies in the field are limited or yield data that are difficult to interpret. Many larval surveys were conducted along the east coast of Canada and the United States (reviews by Stasko 1980; Fair 1980; Fogarty 1983), but few of those were in SWNS. Until the mid 1970's, only 12 larvae were captured offshore of SWNS by neuston net (397 tows) (Stasko 1977). Analysis of the 1976 samples from a Scotian Shelf fish-eggs-and-larvae survey yielded 142 lobster larvae (Stasko 1977). During a 2 years (1977 and 1978) intensive larval survey (1,838 tows) at inshore and offshore stations, 2,325 larvae, including all four stages, were captured primarily in neuston tows (Stasko and Gordon 1983).

A program to develop methods of capturing newly settled Stage IV larvae was initiated in 1982. As a part of this study, the authors attempted to identify areas with

high planktonic concentrations of Stage IV larvae. These sites would then be used to test passive and active collection methods for settled stages. This study incidentally collected other larval stages, and by replication of Stasko's and Gordon's (1983) stations provided comparisons.

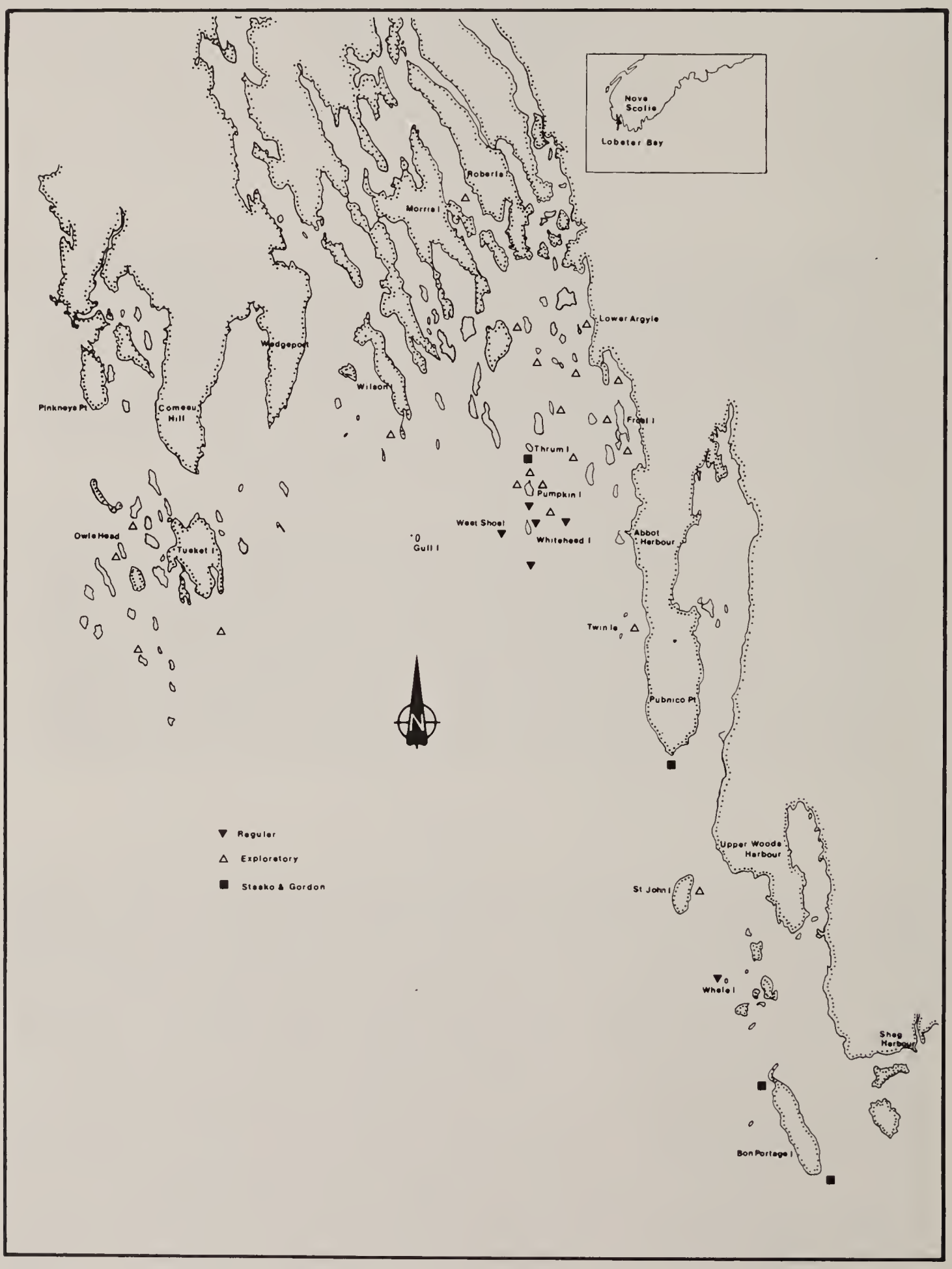

Fig 1 Map of the study area and location of sampling stations in the 1983 *Homarus americanus* larval survey of Lobster Bay, Yarmouth Co., N.S.

Materials and Methods

Lobster larvae were sampled for a period of 10 weeks (July 6 to September 13, 1983) in SWNS. The survey area extended from the Tusket Islands to Bon Portage Island (Fig 1). The majority of the sampling was conducted in Lobster Bay. It is a shallow bay (depth < 20 m) with rocky shallows gently sloping to sand or mud bottom (MacFarlane 1964). The tides are semi-diurnal with a range at spring tides of 4.18 m, creating tidal current flows to 10 km h^{-1} (Anonymous 1982). Islands in the Bay are in a north-to-south direction. Predominant summer winds are from the south to southwest. Intertidal water temperatures ranged between 11.0°C and 16.0°C in July and August 1983 (Dobson and Petrie 1985). Four categories of stations (Table I) were sampled:

1) *Regular stations:* These were located around Whitehead Island and Whale Island. Whale Island was chosen due to knowledge gathered during previous studies and Whitehead Island due to initial success in capturing lobster larvae in that area. These stations were sampled weekly. Tows were generally made during the day, but occasionally at night.

2) *Night stations:* Thirteen percent of all tows (127) were made during the night; all but two night tows were around Whitehead Island.

Table I Location and depth of towing stations in Lobster Bay, Yarmouth Co., N.S., during 1983.

Station	Latitute (north)	Longitude (west)	Depth range (m)
Bon Portage - north	43°29′02″	65°46′04″	1 to 12
Bon Portage - south	43°27′04″	65°44′04″	1 to 10
St. Ann Point	43°35′02″	65°48′02″	2 to 10
Thrum Is.	43°41′03″	65°52′00″	2 to 13
Whale Is.	43°31′02″	65°47′01″	1 to 10
Whitehead Is. - channel	43°40′03″	65°52′00″	8 to 11
Whitehead Is. - west	43°40′01″	65°52′02″	5 to 10
Whitehead ledge	43°39′08″	65°51′00″	3 to 13
West Shoal	43°40′00″	65°52′07″	4 to 6
1.6 km south - Whitehead Is.	43°38′06″	65°52′01″	16 to 18
Pumpkin Is. - east	43°40′05″	65°51′06″	5 to 11
Pumpkin Is. - west	43°40′08″	65°52′03″	5 to 12
Twin Is.	43°37′09″	65°49′03″	4 to 13
Camp Is. to Black ledge	43°42′06″	65°50′08″	2 to 14
Frost Is. - south	43°41′03″	65°49′08″	2 to 7
Birch Is. - north	43°43′05″	65°51′08″	2 to 6
Black ledge to Little Gooseberry Is.	43°42′01″	65°51′02″	2 to 8
Argyle Sound	43°42′06″	65°49′05″	2 to 6
Robert Is. - west	43°46′03″	65°53′09″	1 to 5
Bond Is. - channel	43°43′07″	65°51′07″	5 to 10
Pumpkin Is. to Big Gooseberry Is.	43°40′09″	65°51′05″	5 to 12
Wilson Is. to Eastern Bar	43°41′02″	65°55′06″	2 to 7
Owl's Head Is.	43°39′02″	66°03′02″	2 to 5
Deep Cove Is.	43°39′09″	66°02′04″	10 to 24
Half Bald Is.	43°37′00″	66°02′05″	5 to 13
Old Woman buoy to 91Y buoy	43°37′05″	65°59′00″	2 to 12
St. John Is. - east	43°32′06″	65°47′06″	1 to 3
Big Gooseberry Is. to Frost Is.	43°42′00″	65°50′00″	5 to 15
Lr. Argyle Shore to Nanny Is.	43°44′00″	65°50′05″	2 to 5
Whitehead Ledge to Pumpkin Is.	43°40′00″	65°51′06″	5 to 13
Pumpkin Is. to Thrum Is.	43°40′09″	65°52′01″	5 to 10

3) *Stasko's and Gordon's stations:* Four inshore stations of the Stasko and Gordon (1983) SWNS surveys were sampled weekly. The stations were: Thrum Island, St. Ann Point, Bon Portage north, and Bon Portage south (Fig 1).

4) *Exploratory stations:* These were used to discover other areas of larval concentration. They were spread throughout the study area but only sampled once or twice (Fig 1).

Harding et al. (1982) and Fair (1980) mentioned possible concentration of larvae by various current phenomena. Thus, whenever possible, tows were made through eddies, slicks, foam lines, and windrows.

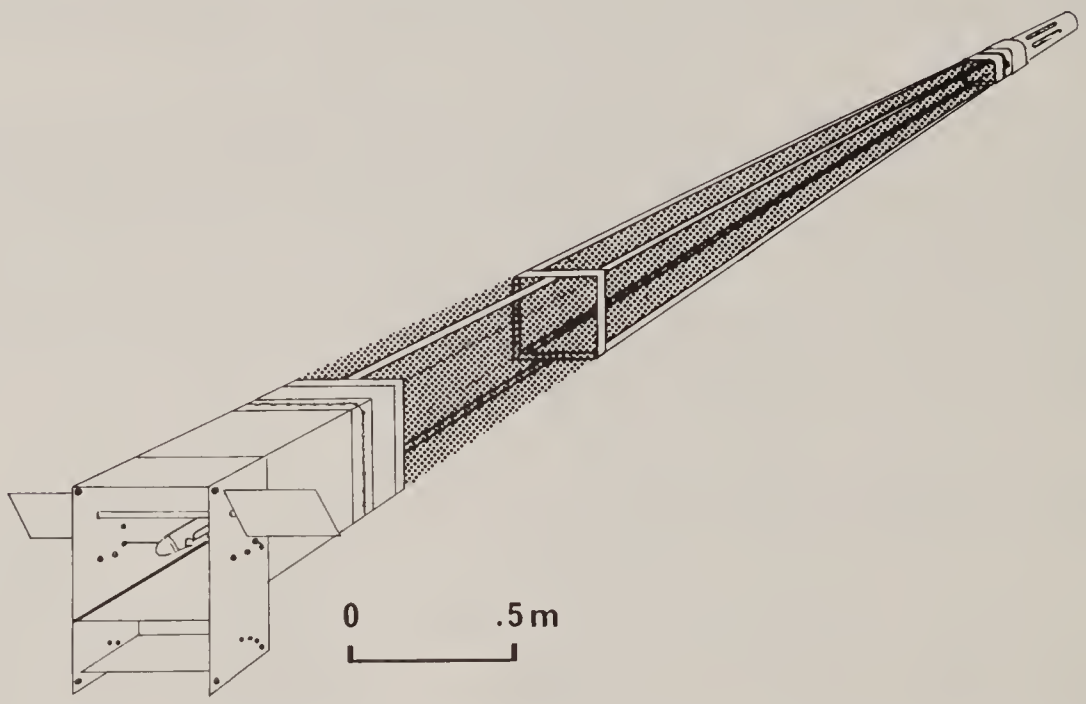

Fig 2 Neuston sampler, as described by Sameoto and Jaroszynski (1969).

Two neuston samplers (Sameoto and Jaroszynski 1969), each 0.4 m x 0.4 m with Nitex 502 μm mesh nets, were towed for 30 min (Fig 2). The samplers were towed on each side of a 6 m Cape Islander-style boat on 2 m booms (Fig 3). Tow speed was adjusted to allow the sampler frame to break the surface of the water. Each net sampled the top 0.4 m of the water column and filtered an average of 305 m^3 of water during a 30 min tow at *ca.* 2 knots. The amount of water filtered was determined by averaging readings obtained from a General Oceanic Incorporated flowmeter installed on one sampler. Six tows were completed with only one net (i.e. half tows) as a result of damage to the other net; the remaining 124 tows were made with two nets.

The towing stations were close to islands or the mainland (less than 2 km) and over shallow waters (less than 20 m). Stations (Table I) were located by a combination of landmarks, water depth, and radar fixes. To restrict the sampled area, the course was reversed 15 min into the tow or at the limit of a landmark. During each tow, surface temperature, wind speed/direction, and cloud cover were recorded. After the tow, samples from each net were examined separately without visual aids. Large pieces of vegetation were checked for larvae, rinsed, checked again, then discarded. Port and starboard samples were separately preserved in 5% buffered formaldehyde solution. Later, samples were sorted for lobster larvae, representative crustaceans, and fish larvae. Lobsters were inspected using a dissecting microscope and staged according to Herrick (1911). Port and starboard samples for each tow were combined for data analysis, as the towing pattern took the nets through the same body of water usually more than once.

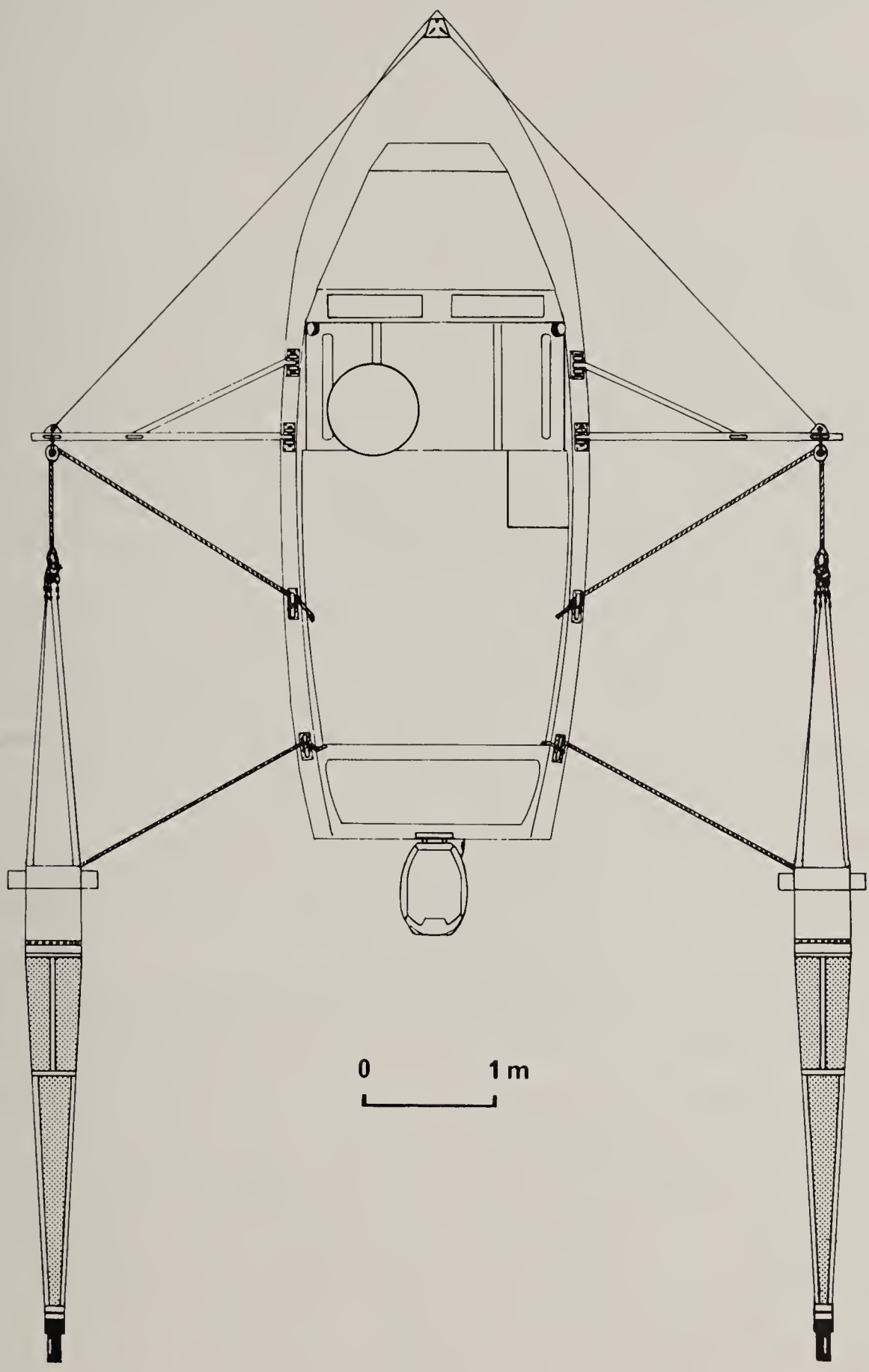

Fig 3 Towing configuration for two neuston samplers from a 6 m outboard-powered vessel.

Results

In 28 sampling days between July 6 and September 13, 1983, 171 larvae were caught, an average of 2.21 larvae 1000 m^{-3} of water filtered. The composition of the catch was as follows.

Larval stage	Number
I	159 (93%)
II	4 (2%)
III	1 (1%)
IV	7 (4%)
A	Total: 171

Regular stations At Whale Island, one Stage I was captured in 13 tows. However, around Whitehead Island larvae were consistently captured. Forty-nine percent of the towing took place around Whitehead Island, and yielded 89% of all the larvae captured. Although larvae were difficult to locate in Lobster Bay on this survey, on the first sampling day (July 6, 1983) at Whitehead Island, four Stage I larvae were caught in a tow with one net. The most successful sampling day was July 20 at stations around Whitehead Island; 51% of all larvae were caught (20.61 larvae 1000 m^{-3}) (Table II). During the sampling period, seven of the tows around Whitehead Island were made in surface phenomena. They yielded ten larvae (2.34 larvae 1000 m^{-3}).

Night stations All successful night towing (19:00-07:00 h) was around Whitehead Island; two exploratory night tows yielded no larvae. Night towing at Whitehead Island captured 2.84 larvae 1000 m^{-3}, while day towing captured 4.38 larvae 1000 m^{-3}, approximately 1.5 times more. But night tows were not as frequent as day tows. If we compare results on a weekly basis, night towing was successful (Table III). The majority of the night tows took place August 25-26, 1983, during continuous day-night towing around Whitehead Island. The six "day" tows yielded one Stage I larvae (0.27 larvae 1000 m^{-3}), while the "night" tows yielded 22 larvae (3.28 larvae 1000 m^{-3}) (Fig 4). Of these, two day and one night tow were made in surface phenomena and yielded one and three Stage I larvae, respectively. The largest number of larvae (11) were captured at twilight (Fig 4). It was also during this period that the only Stage III larva was captured (Fig 4). Two of the four Stage II and four of the seven Stage IV larvae found in the surveys were captured in night tows.

Exploratory stations Five Stage I larvae occurred in 19 tows (0.43 larvae 1000 m^{-3}) (Table II). This was the least successful category of stations, despite the range of sites selected, from intertidal at high tide to 24 m of water, over mud, rock, and sand

Table II Absolute numbers of larvae captured, by week and by station categories, in Lobster Bay, Yarmouth Co., N.S., during 1983.

Date (1983)	Regular stations - day					Regulation stations - night				
		Larval Stage					Larval Stage			
	# tows	I	II	III	IV	# tows	I	II	III	IV
July 4-8	0.5	4	0	0	0					
July 11-15	4	9	0	0	0					
July 18-22	7	88	1	0	0					
July 25-29	4	3	0	0	0	1	0	0	0	0
Aug. 1-5										
Aug. 8-12	11	12	0	0	0					
Aug. 15-19	4	3	0	0	0					
Aug. 22-26	7	1	0	0	2	10	16	2	0	4
Aug. 29-Sept. 2	2	1	0	0	0	2	3	0	0	0
Sept. 5-9	4	2	1	0	0	2	0	0	1	0
Sept. 12-16	4	1	0	0	0					
Total:	47.5	124	2	0	2	15	19	2	1	4

Table III Weekly comparisons of 1983 day and night towing at Whitehead Island, Yarmouth Co., N.S. (number of larvae 1000 m^{-3} of water filtered).

Date	Day towing	Night towing
July 25-29	1.23	0
Aug. 22-26	0.70	3.61
Aug. 29-Sept. 2	0.82	2.46
Sept. 5-9	1.23	0.82

substrates. Tows in surface phenomena occurred in 3.5 of 19 exploratory tows yielding two Stage I larvae.

Stasko's and Gordon's stations Replication of Stasko's and Gordon's four inshore stations (Stations 206, 207, 301, 303) found 12 larvae in 30.5 tows (0.64 larvae 1000 m^{-3}) (Table IV). Stasko and Gordon (1983) caught an average of 0.80 larvae 1000 m^{-3} and 2.35 larvae 1000 m^{-3} in 1977 and 1978, respectively.

Results were not exactly comparable because Stasko and Gordon sampled a shallower part of the water column (i.e. 0.15 m versus 0.4 m), at higher speed (i.e. 4 knots versus 2 knots). The tows also covered a larger area than did the tows in this survey, as in this survey the course was reversed half way in the tow. All 3 years, the catch consisted of Stages I and IV larvae exclusively. However, Stage I larvae was dominant in our survey versus Stage IV larvae in Stasko's 1977—1978 surveys. The percent composition of the catch was as follows:

Stage	1977	1978	1983
I	11%	29%	92%
II	0%	0%	0%
III	0%	0%	0%
IV	89%	71%	8%

Seven of the 30.5 tows were made in surface phenomena, yielding four Stage I larvae.

Table II (Cont.) Absolute numbers of larvae captured, by week and by station categories, in Lobster Bay, Yarmouth Co., N.S., during 1983.

Date (1983)	Exploratory stations					Stasko and Gordon stations				
		Larval Stage					Larval Stage			
	# tows	I	II	III	IV	# tows	I	II	III	IV
July 4-8	1	0	0	0	0	0.5	0	0	0	0
July 11-15	2.5	1	0	0	0	4	0	0	0	0
July 18-22	4	3	0	0	0	4	4	0	0	0
July 25-29	2.5	0	0	0	0	2	3	0	0	0
Aug. 1-5										
Aug. 8-12	4	0	0	0	0	4	3	0	0	0
Aug. 15-19	3	1	0	0	0	4	0	0	0	1
Aug. 22-26	2	0	0	0	0	2	1	0	0	0
Aug. 29-Sept. 2							3	0	0	0	0
Sept. 5-9							3	0	0	0	0
Sept. 12-16							4	0	0	0	0
Total:	19	5	0	0	0	30.5	11	0	0	1

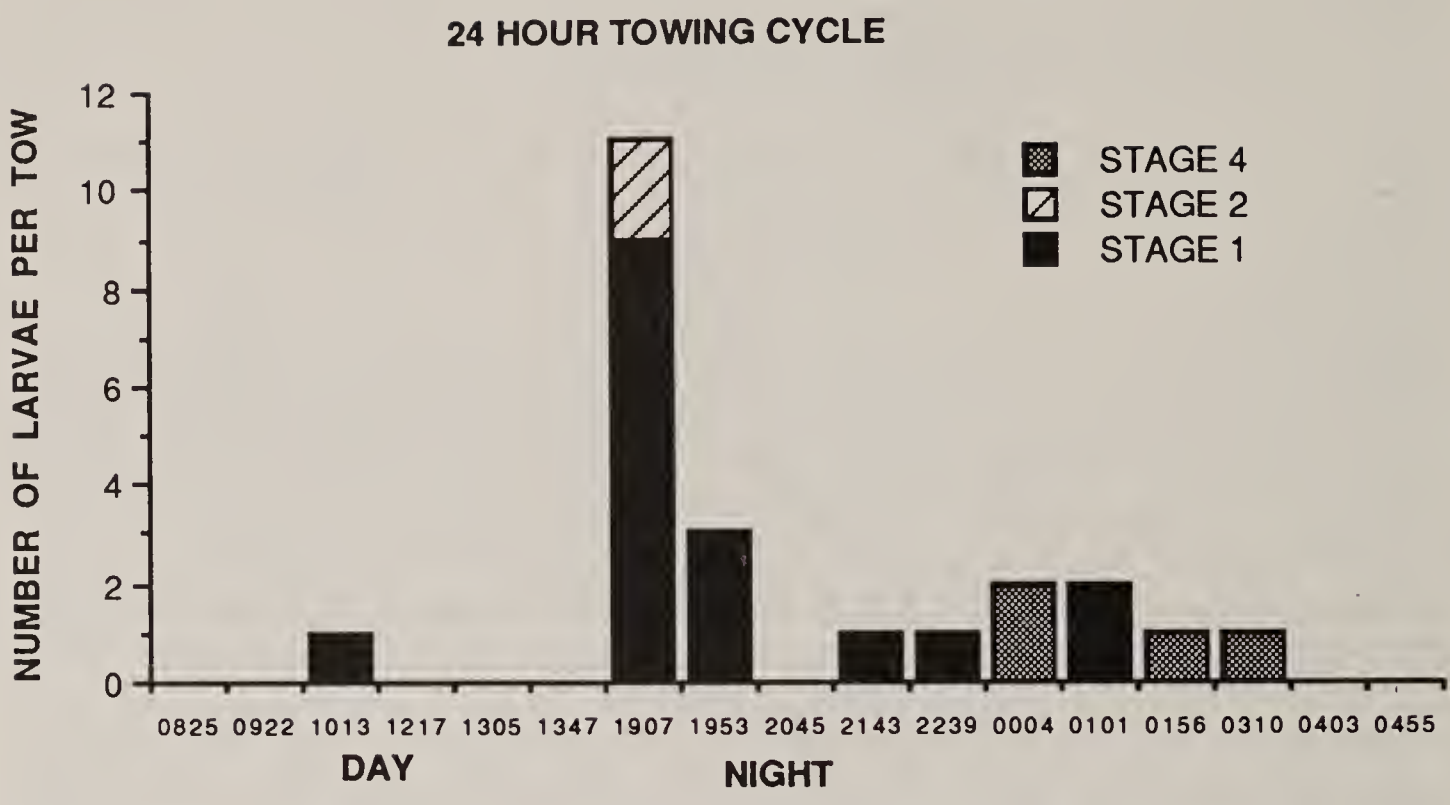

Fig 4 Results of the day-night towing cycle, August 25-26, 1983, absolute numbers caught per tow (two nets).

Discussion

The patchiness of spatial and temporal captures in this survey was not atypical for lobster larval surveys. Frequently one station or one day will yield a large proportion of the total captures in an extensive survey. For example, in Buzzard Bay, Massachusetts, 746 lobster larvae (266.2 larvae 1000 m^{-3}) were collected in one tow, representing 18% of the total catch for that year (Collings et al. 1983). Lux et al. (1983) captured 6,746 larvae in five tows in 1 day (450 larvae 1000 m^{-3}), representing 65% of their total 1979 catch. Part of the problem is a poor understanding of vertical distribution of all larval stages and the influence of behaviour relative to physical phenomena (Harding et al. 1987). Stage IV larvae were found to be equally abundant in Canadian offshore waters both day and night (Harding et al. 1987). Most of our towing was done in the daytime, yet we found few Stage IV larvae. The four stations established by Stasko and Gordon (1983) produced a predominance of Stage IV larvae in 1977 and 1978 but

Table IV Number of lobster larvae 1000 m^{-3} of water filtered in neuston gear at Stasko's and Gordon's stations.

	Station 206 - Bon Portage north					Station 207 - Bon Portage south				
	Larval stage					Larval stage				
Year	I	II	III	IV	Total #	I	II	III	IV	Total #
1977	0	0	0	0.47	1	0.37	0	0	1.49	5
1978	0	0	0	4.15	20	1.68	0	0	0.56	12
1983	0.47	0	0	0	2	0.61	0	0	0	3

Note 1977 and 1978 neuston nets sampled the top 0.15 m of the water column, and filtered 535 m^3 of water during a 30 min tow at 4 knots.

1983 neuston nets sampled the top 0.4 m of the water column, and filtered 610 m^3 of water during a 30 min tow at ca. 2 knots.

Stage I larvae in 1983. Harding et al. (1987) caught Stage I larvae in offshore waters, mostly between 15 to 30 m during the day and usually above 10 m at night.

The predominance of Stage I larvae in our survey cannot be explained by diel towing procedure, as towing was done mainly during the day. However, in the 24-hour tow series 16 Stage I larvae were caught in ten night tows, and only one in six day tows. The 14 inshore stations of Stasko and Gordon (1983) between Cape Sable Island and Pinkneys Point in 1977 yielded 85% Stage I larvae, and the converse in 1978, 28% Stage I. Diel migration cannot explain these differences, as daylight towing was used in both years for inshore stations. These data suggest annually cyclic and spatial factors are more predominant in the near shore than in the offshore environment. A survey of eight stations *ca.* 8 km apart in Cape Cod Bay found greater than 20 Stage IV larvae 1000 m^{-3} in seven stations and only 2.6 Stage IV larvae 1000 m^{-3} in the eighth station (Matthiessen and Scherer 1983). Similarly, abundance of all stages in shallow waters can fluctuate dramatically between years; in Buzzard Bay mean larval density ranged from 8.6-15.9 1000 m^{-3} in a 3 years series (1976-1978) (Collings et al. 1983). Spatially adjunct water bodies had an extreme mean range, from 1.3 to 15.8 larvae 1000 m^{-3} within 1 year (Collings et al. 1983).

The limited area covered by this survey's towing technique enabled the identification of areas of concentration on the scale of 0.5 km. As a result Whitehead Island was selected as a station early in the survey and produced consistent results. Positive results occurred in 54% of tows at Whitehead Island versus 23% at Stasko's and Gordon's stations and 8% at Whale Island. Larvae were collected from Whitehead Island every week of the survey. The consistency of capture in this area may be due to several factors relating to source of larvae and physical factors.

Lobster Bay is an area of high lobster abundance; all the shoals and foreshores yield large catches. In 1983, inshore lobster landings were 1536 metric tons (the Department of Fisheries and Oceans' Statistical District 33). Egg-bearing females are present in the Bay during summer months (D.E. Graham[1]).

The shallows of Whitehead Island contain a part of this high lobster abundance. High-speed tidal currents, 2-4 km h^{-1}, pass around Whitehead Island; and the position of land mass and shoals may carry or concentrate larvae from near and distant sources more frequently through this area than at other stations.

However, it is not possible to distinguish larval sources. Stage IV larvae appeared 4 weeks after Stage I larvae in near-shore waters of Lobster Bay. The period of time

[1]D.E. Graham, Biological Sciences Branch, Department of Fisheries and Oceans, St. Andrews, N.B. E0G 2X0.

Table IV (Cont.) Number of lobster larvae 1000 m^{-3} of water filtered in neuston gear at Stasko's and Gordon's stations.

	Station 301 - St. Ann Point					Station 303 - Thrum Island				
	Larval stage					Larval stage				
Year	I	II	III	IV	Total #	I	II	III	IV	Total #
1977	0	0	0	0.31	1	0	0	0	0.62	2
1978	0.37	0	0	1.12	8	0.56	0	0	1.12	9
1983	1.01	0	0	0	4	0.36	0	0	0.18	3

Note 1977 and 1978 neuston nets sampled the top 0.15 m of the water column, and filtered 535 m^3 of water during a 30 min tow at 4 knots.

1983 neuston nets sampled the top 0.4 m of the water column, and filtered 610 m^3 of water during a 30 min tow at *ca.* 2 knots.

between the appearance of Stage I and Stage IV larvae was approximately 2-3 weeks in 1977 and 1978 (Stasko and Gordon 1983). A minimum of 3 weeks is required at 14.5°C to reach Stage IV (MacKenzie 1985). A local source of larvae is possible for our study but less likely in the Stasko and Gordon survey due to the lack of degree days.

It is not justified to create any theories concerning diel and vertical distribution patterns from our limited data base. However, it is clear that the shallow near-shore environment with extreme tidal mixing must be examined directly, and assumptions derived from offshore larval studies cannot be easily applied.

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References

Anonymous. 1982. Canadian tide and current tables. Vol. 1, 1983. Dept. Fish. and Oceans-Can. Hydrogr. Serv.

Collings, W.S., Cooper-Sheehan, C., Hughes, S.C., and **Buckley, J.L.,** 1983. The spatio-temporal distribution of American lobsters, *Homarus americanus,* larvae in the Cape Cod Canal and approaches, p. 35-40. *In* M.J. Fogarty (ed.) Distribution and abundance of American lobster, *Homarus americanus,* larvae: New England investigations during 1974-1979. NOAA Tech. Rep. NMFS SSRF-775:64 p.

Dobson, D. and **Petrie, B.,** 1985. Long-term temperature monitoring program 1984, Scotia-Fundy, Gulf Regions. Can. Data Rep. Hydrogr. Ocean Sci. 35:vii + 691 p.

Fair, J.J., Jr. 1980. U.S. surveys of lobster larvae. *Can. Tech. Rep. Fish. Aquat. Sci.* 932:153-155.

Fogarty, M.J. 1983. Distribution and relative abundance of American lobster, *Homarus americanus,* larvae: A review, p. 3-8. *In* M.J. Fogarty (ed.) Distribution and relative abundance of American lobster, *Homarus americanus,* larvae: New England investigations during 1974-1979. NOAA Tech. Rep. NMFS SSRF-775:64 p.

Harding, G.C., Vass, W.P., and **Drinkwater, K.F.** 1982. Aspects of larval American lobster (*Homarus americanus*) ecology in St. Georges Bay, Nova Scotia. *Can. J. Fish. Aquat. Sci.* 39:1117-1129.

Harding, G.C., Pringle, J.D., Vass, W.P., Pearre Jr., S. and **Smith, S.J.** 1987. Vertical distribution and daily movements of larval lobsters *Homarus americanus* over Browns Bank, Nova Scotia. *Mar. Ecol. Prog. Ser.* 41:29-41.

Herrick, F.H. 1911. Natural history of the American lobster. *Bull. U.S. Bureau Fish.* 29:149-408.

Lux, F.E., Kelly, G.F., Wheeler, C.L., 1983. Distribution and abundance of larval lobsters (*Homarus americanus*) in Buzzards Bay, Massachusetts, during 1976-1979, p. 29-33. *In* M.J. Fogarty (ed.) Distribution and relative abundance of American lobster, *Homarus americanus,* larvae: New England investigation during 1974-1979. NOAA Tech. Rep. NMFS SSRF-775:64 p.

MacFarlane, C.I. 1964. A comparison of two marine drumlin regions in Nova Scotia, p. 240-247. *In* A.D. De Verville and J. Feldmann (ed.) Proceedings of the Fourth International Seaweed Symposium.

MacKenzie, B. 1985. Temperature considerations of larval lobster (*Homarus americanus* Milne Edwards) ecology in waters off southwestern Nova Scotia. M.Sc. Thesis, Dal. Univ.: 106 p.

Matthiessen, G.C. and **Scherer, M.D.,** 1983. Observations on the seasonal occurrence, abundance and distribution of larval lobsters (*Homarus americanus*) in Cape Cod Bay, p. 41-46. *In* M.J. Fogarty (ed.) Distribution and relative abundance of American lobster, *Homarus americanus,* larvae: New England investigations during 1974-1979. NOAA Tech. Rep. NMFS SSRF-775:64 p.

Pezzack, D.S. and **Duggan, D.R.,** 1983. The Canadian offshore lobster (*Homarus americanus*) fishery - 1971-1982. Int. Coun. Explor. Sea C.M. 1983/K:34.

Pezzack, D.S. and **Pringle, J.D.** 1986. Gulf of Maine area lobster management areas, and suggestions on stock structure. Can. Atl. Fish. Sci. Adv. Comm. Res. Doc. 86/65.

Sameoto, D.D. and **Jaroszynski, L.O.,** 1969. Otter surface sampler: A new neuston net. *J. Fish. Res. Board Can.* 26:2240-2244.

Stasko, A.B. 1977. Lobster larvae on the Scotian Shelf. Can. Atl. Fish. Sci. Adv. Comm. Res. Doc. 77/31.

Stasko, A.B. 1978. Inshore-offshore SW Nova Scotia lobster stock interaction: A hypothesis. Can. Atl. Fish. Sci. Adv. Comm. Res. Doc. 78/37.

Stasko, A.B. 1980. Lobster larval surveys in Canada. Can. Tech. Rep. Fish. Aquat. Sci. 932:157-169.

Stasko, A.B., and **Gordon, D.J.,** 1983. Distribution and relative abundance of lobster larvae off southwestern Nova Scotia, 1977-1978. Can. Tech. Rep. Fish. Aquat. Sci. 1175:iii + 23 p.

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COMMUNITY ANALYSIS OF FISH POPULATIONS IN HEADWATER LAKES OF NEW BRUNSWICK AND NOVA SCOTIA

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Five fish community assemblages, based upon surveys with gill-nets, were identified in Maritime headwater lakes. These were (1) brook trout only, (2) brook trout-white sucker, (3) multispecies with white sucker, yellow perch and brown bullhead most frequent members, (4) yellow perch only and (5) multispecies with smallmouth bass and/or white perch. The two lake physico-chemical parameters most important in determining fish community were pH and lake area. Brook trout-only lakes had the least area, while yellow perch-only lakes were the most acidic (mean pH of 4.60). It is suggested that lakes containing only yellow perch may have once contained assemblage (3), but have become too acidic for any but yellow perch.

Cinq groupements de poissons, bases sur des relevès au moyen de filets, ont été identifiées dans quelques lacs eaux—souches des Provinces Maritimes. Ceux-ci comprenaient (1) l'omble de fontaine seul, (2) l'omble de fontaine et le meunier noir, (3) plusieurs espèces comprenant le meunier noir, la perchaude et la barbotte brune en majorité, et (4) la perchaude seule. Les deux paramètres physico-chimiques de plus grande importance quant aux groupements de poissons sont le pH et la superficie des lacs. Les lacs avec l'omble seul ont la moindre superficie, tandis que les lacs contenant la perchaude seule sont les plus acidiques (un pH moyen de 4.60). On suggère que les lacs contenant seulement la perchaude contenaient dans le passé le groupement (3), mais sont devenus trop acidiques pour maintenir aucune espèce autre que la perchaude.

Introduction

The possible loss of fish populations has been a primary concern of scientists studying the ecological effects of acid precipitation. The effects of acidification on fish populations are most easily discerned where adequate historical data on both fish populations and water chemistry exists. Beamish and Harvey (1972) reported acidification of La Cloche Mountain lakes in Ontario and associated losses of fish species. Schofield (1976) associated losses of fish populations in lakes in the Adirondack Mountains with acidification of these waters. In Scandinavia, similar relationships have been proposed (Muniz *et al.*, 1976; Snekvik, 1974). Even in the absence of good historical data, lake surveys are useful in examining how physico-chemical parameters of lakes can influence fish populations. Thus, concern about lake and stream acidification has resulted in many surveys of fish populations with concomitant water chemistry analyses. This report presents results of the Maritime portion of a survey of headwater lakes in eastern Canada done to estimate the potential for lake acidification in this region (Kelso *et al.*, 1986).

Recurrent questions asked by those who perform analyses on data sets from large numbers of lakes are whether fish populations can be classified into a few general assemblage types and, if so, whether such assemblages are related to any physico-chemical properties of the lakes where they occur.

Several trends have been shown to consistently occur in published accounts of lake survey data. Species richness (i.e., number of species) tends to increase with increasing lake area (Haines *et al.*, 1986; Eadie *et al.*, 1983; Johnson *et al.*, 1977; Rahel, 1984). MacArthur and Wilson (1967) formulated a general theory to explain the relationship between species richness and island size, wherein under equilibrium conditions a balance is struck between immigration of new species and extinction of those present. The slope relating species richness to lake area has been found to lie within the range of values published for island groups (Matuszek and Beggs, 1988). Whether

or not the species richness-lake area relationship reflects immigration-extinction phenomena or reflects increased habitat diversity in larger lakes is a matter of discussion (e.g. Eadie *et al.*, 1983).

Another observed trend is that associated with lake eutrophication (Colby *et al.*, 1972; Leach *et al.*, 1977), where salmonid communities of low diversity are replaced successively by centrarchid and cyprinid communities as eutrophication progresses. Rahel (1984), in an analysis of lakes at various stages of dystrophication, could separate three distinct fish assemblages. An *Umbra*-yellow perch assemblage was characteristic of the most acidic lakes. Two other assemblages "centrarchid" and "cyprinid" were related to lake size, and low winter oxygen tension, respectively. Van Leuven and Oyen (1987), in an analysis of Netherlands' ponds and lakes, also found *Umbra* to be characteristic of very acidic waters. Haines *et al.* (1986) in an analysis of lakes in the northeastern U.S. were able to distinguish three assemblages: "sunfish-sucker," "cyprinid," and "depauperate". The important lake parameters separating those assemblages were lake area, depth and ionic strength. Factors associated with acidity were important in distinguishing fishless lakes. Other studies have found that "fishless" lakes occur frequently at low lake pH (Kelso *et al.*, 1986). It is possible that fish community response to natural dystrophication (Rahel, 1984) is distinct from the response to anthropogenic acidification. The latter process is much more rapid, so that changes in fish assemblages may lack sufficient time to stabilize.

In this paper we analyze the information on fish species collected from 145, mostly headwater, lakes in New Brunswick (N.B.) and Nova Scotia (N.S.) by gill-netting.

Materials and Methods

The lakes selected for the survey were situated on granitic or metamorphic bedrock materials known to provide low buffering capacity. Lake and catchment areas were measured and catchment: lake ratios calculated. Lakes were selected from this catalogue, based upon low catchment: lake area ratios and relative lack of human disturbance. Most were headwater lakes; however, a few higher order lakes were included and some clustering of selected lakes was done to minimize helicopter expenses.

Information on lake physico-chemical parameters and fish species present was collected in July-August of 1981 and 1982. The methods used in collection of water samples, chemical analyses, and estimation of lake physical parameters are described in Peterson *et al.* (1986), and Kelso *et al.* (1986).

The multifilament experimental gillnets used were of 63.7 m total length, 1.8 m deep, and consisted of seven panels with mesh sizes of 3.8, 5.1, 6.4, 7.6, 8.9, 10.2, and 12.7 cm, respectively. The nets were fished overnight. The nets were fished on the surface, set offshore from a rocky point (where possible) with the smallest mesh size nearest shore.

Since only gillnets were used to sample the lakes, the analysis is confined to specimens (and species) large enough to be collected. Thus several small species (e.g. banded killifish, sticklebacks) are excluded from the analysis. Several other species were excluded due to very low sampling efficiency (e.g. American eel) or very low frequency of collection (e.g. alewife, striped bass).

Lake physico-chemical variables were transformed when necessary to normalize the distributions, with skewness and kurtosis values used to determine the most appropriate transformation (SPSSX, 1986). Lake chloride, lake SO_4^{2-} and pH required no transformation, while the other parameters utilized were transformed as follows: K^+, and DOC (log X +1); lake area (log X); and depth (square root).

Statistical analyses. A detrended correspondence analysis (DECORANA; Hill, 1979a) was utilized to ordinate lakes on the basis of species sampled from them. Abundance of species was also taken into consideration in the analysis by the use of "pseudo-species." A species could be represented by up to four pseudo-species, thus providing a method of weighting species importance according to abundance. Abundances of 1-4 were assigned a pseudospecies value of 1, 5-14 a value of 2, 15-24 a value of 3 and >24 a value of 4. The detrended correspondence analysis technique is a reciprocal averaging technique with "detrended" orthogonal axes to eliminate any "horseshoe" effect due to compression of the extremes of calculated environmental axes (Gauch, 1982). The results of this lake by species ordination was tabulated, with lakes ranked in order of ordination scores, and the tabulated lake sequence separated into "assemblages" by visual inspection. A polythetic divisive cluster analysis of the data was also performed (TWINSPAN; Hill, 1979b) and clusters delineated were used to assist in separation of the fish communities into assemblages. An eigen value of 0.3 was used as the criterion to terminate level of clustering. The "assemblages" so designated were correlated with various physico-chemical parameters by a multiple discriminant function analysis (MDA:SPSSX). In this analysis, linear combinations of the environmental variables were used to discriminate among groups of lakes, with all variables entered into the functions simultaneously (Green and Vascotto, 1978). The discriminant functions used were based on the statistical significance ($p \leq 0.05$) of the Wilks' lambda statistic. The discriminant functions utilizing the physico-chemical parameters were then used to predict group membership of each lake with respect to species assemblage; thus the accuracy of the functions in allocating group membership could be assessed.

Fish species were also ordinated on the basis of the lakes where they were found, using detrended correspondence analysis — the inverse of the lake ordination procedures described above.

Results

Ordination of lakes on the basis of species present. The lakes were ordinated along four orthogonal axes by detrended correspondence analysis, based on fish species present, with eigen values for axes 1-4 of 0.854 (48% of the variance), 0.556 (31%), 0.254 (14%) and 0.109 (6%). Since axis 1 accounted for nearly 50% of the variance, the various lakes were ranked according to their position along this axis (App. 1). The two most obviously defined assemblages were formed by lakes from which only *Salvelinus fontinalis* (brook trout) were netted (assemblage 1) and those from which only *Perca flavescens* (yellow perch) were netted (assemblage 4). These assemblages were also well defined in the cluster analysis as clusters A and H. The cluster analysis separated the yellow perch assemblage into two clusters (D, H) based upon numbers of perch netted. It seems reasonable to amalgamate them as one assemblage as was done by the ordination scores. The fish communities of the remaining lakes form a graded continuum with various species forming successive maxima in abundances as one proceeds along axis 1. *Catostomus commersoni* (white sucker), *Ictalurus nebulosus* (brown bullhead) and yellow perch form successive maxima in the central portion of the axis. *Morone americanus* (white perch), *Esox niger* (chain pickerel) and *Micropterus dolomieui* (smallmouth bass) have the lowest scores along axis 1 and form the opposite end of the axis from brook trout. Assemblage 2, containing brook trout and white sucker, but usually lacking brown bullhead or yellow perch, forms a fairly discrete segment of the continuum (mainly cluster B). We also delineated assemblage 5 from 3, based on high abundances of one or more of white perch, chain pickerel and smallmouth bass.

The detrended correspondence analysis, as with all ordination procedures averages species along an undefined "environmental" gradient, such that species "abundance" rises and falls in a normal curve exhibiting "optima" at various points along the gradient. The analysis is successful in this regard for the major species: brook trout, white sucker, brown bullhead, *Semotilus corporalis* (fallfish) and yellow perch.

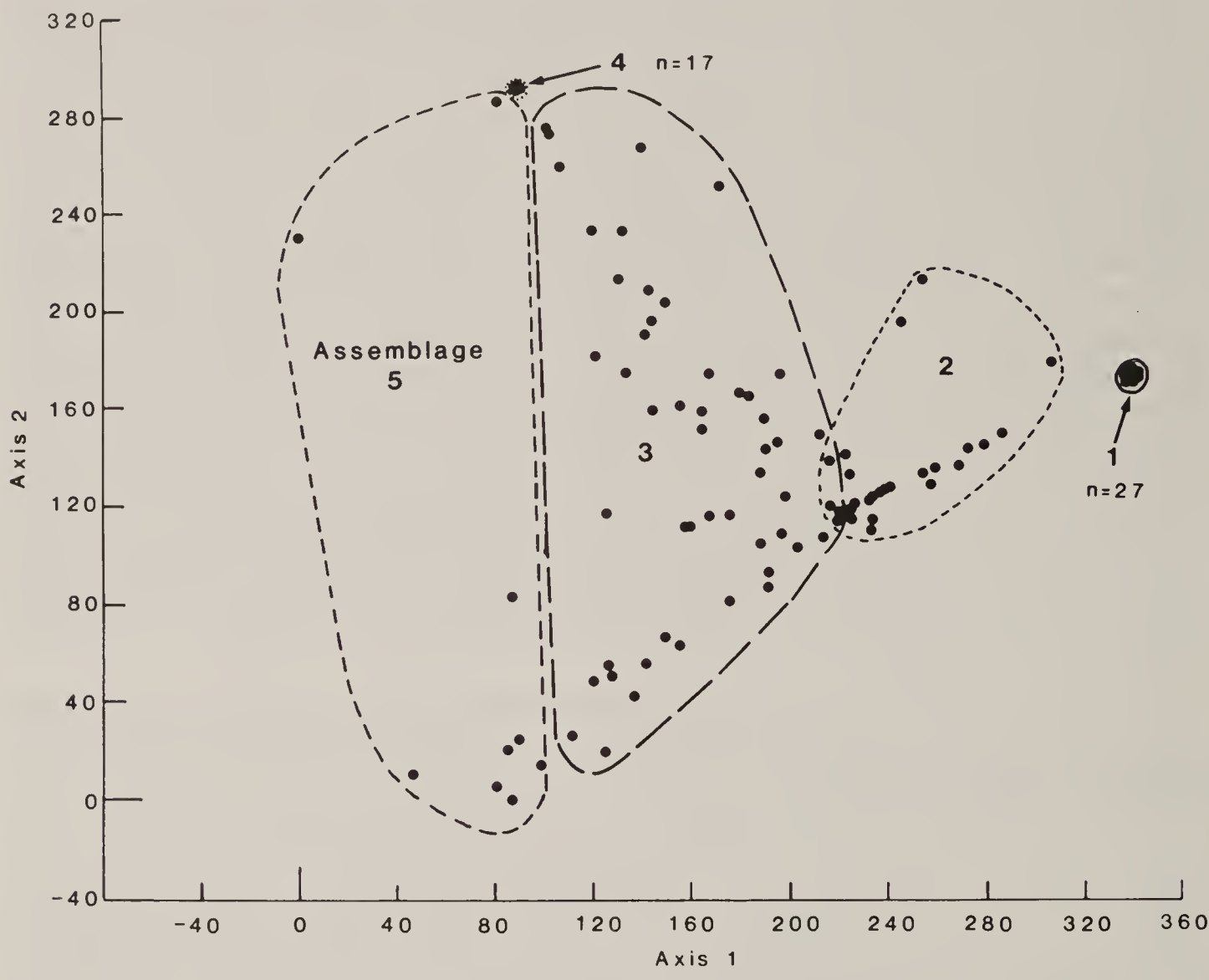

Fig 1 Each lake surveyed is positioned, according to the fish species present, on a grid of the first two ordination axes, as determined by detrended correspondence analysis. Axis calibrations are ordination scores.

We have also plotted the position of the lakes on a grid of the first two ordination axes, which combined account for 80% of the total variance (Fig 1). Assemblages 1 (brook trout only) and 4 (yellow perch only) form distinct clusters. Assemblages 3 and 5, however, become broadly smeared along axis 2, indicating considerable heterogeneity along the axis. Thus most of the variance accounted for by axis 2 is associated with assemblages 3 and 5. Lakes of assemblages 3 and 5 with low scores on axis 2 are dominated by a white perch-chain pickerel association. High scores along axis 2 in assemblages 3 and 5 are associated with either yellow perch or smallmouth bass. Variance accounted for by axes 3 and 4 (not shown in this paper) are mainly associated with separating fallfish from brown bullhead (axis 3) and smallmouth bass from yellow perch (axis 4). We may summarize the results of the detrended correspondence analysis by saying that two lake clusters with assemblages of low species richness (brook trout only and yellow perch only) are defined. Another large cluster of lakes is characterized by a highly species-rich assemblage of which white sucker, brown bullhead, fallfish and yellow perch are most numerous. Other common species in this assemblage are *Lepomis gibbosus* (pumpkinseed sunfish), *Notemigo-*

rus crysoleucas (golden shiner) and chain pickerel. The golden shiner is probably much more common in this assemblage than is indicated by our data. Brook trout occur occasionally in assemblage 3.

Assemblage 2, intermediate between assemblages 1 and 3, is characterized by the presence of brook trout and white sucker - other common species of assemblage 3 occurring infrequently. Lake chub are probably more common in lakes with assemblage 2 than our data indicate.

Assemblage 5 may represent one end of the gradient of assemblage 3 with white perch, chain pickerel and smallmouth bass frequently co-occurring. At the other extreme, assemblage 3 grades into assemblage 2 - the white sucker, brook trout assemblage.

One can also ordinate the fish species, instead of the lakes, on the same ordination axes in the DECORANA program (Fig 2). Comparing Figs 1 and 2, we can see that brook trout and yellow perch occupy the positions of lake assemblages 1 and 4, respectively on the grid of ordination axes 1 and 2. White suckers, *Salmo salar* (landlocked salmon) and *Couesius plumbeus* (lake chub) occupy the region of assemblage 2. The region of assemblage 3 is occupied by brown bullhead, fallfish, pumpkinseeds and golden shiners; chain pickerel, white perch and smallmouth bass

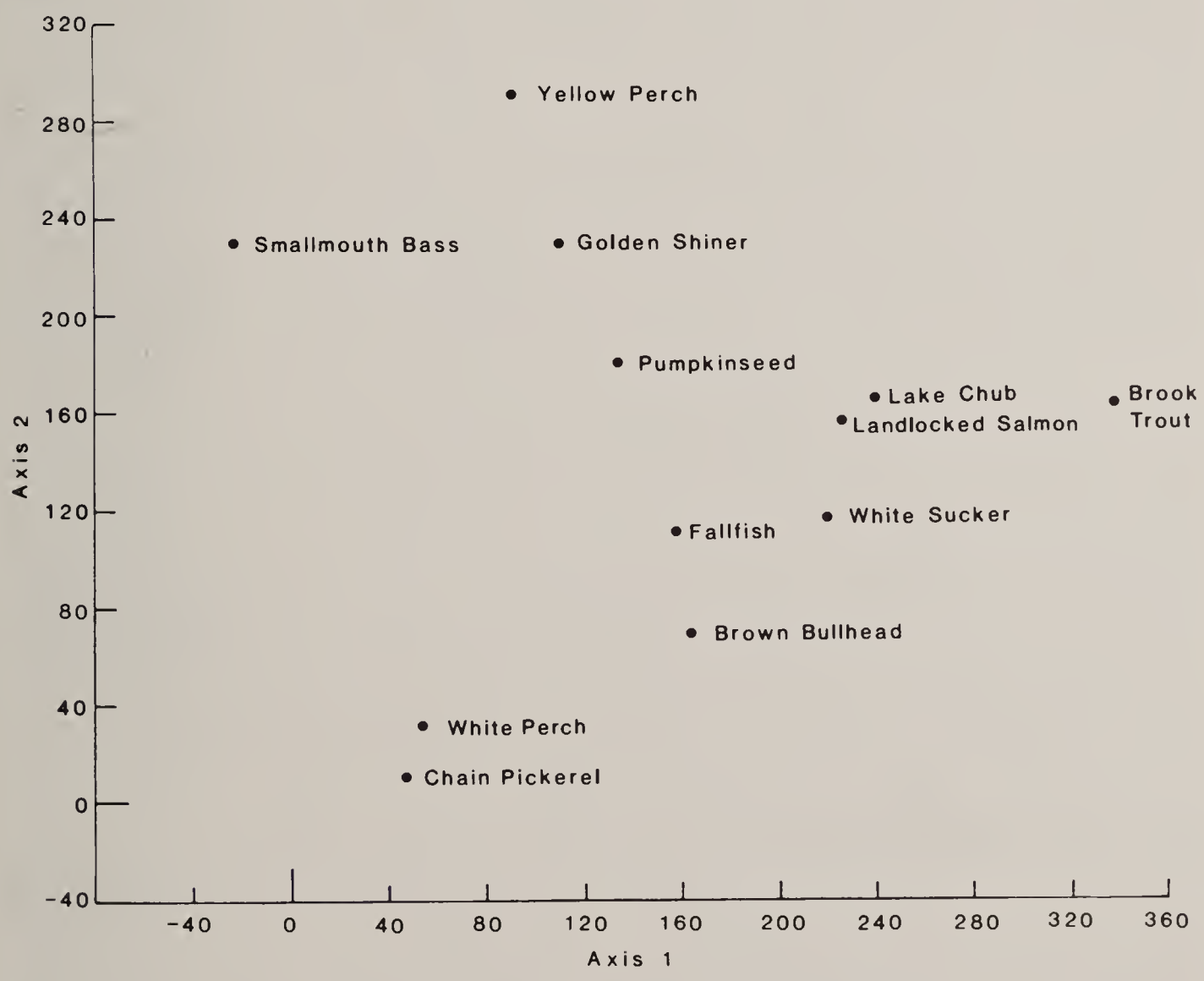

Fig 2 The location of each of the fish species used in the detrended correspondence analysis are arrayed in relation to the first two ordination axes (the same axes on which lakes are arrayed in Fig 1).

Fig 3 Geographic location of lakes containing fish assemblages 1, 4 and 5.

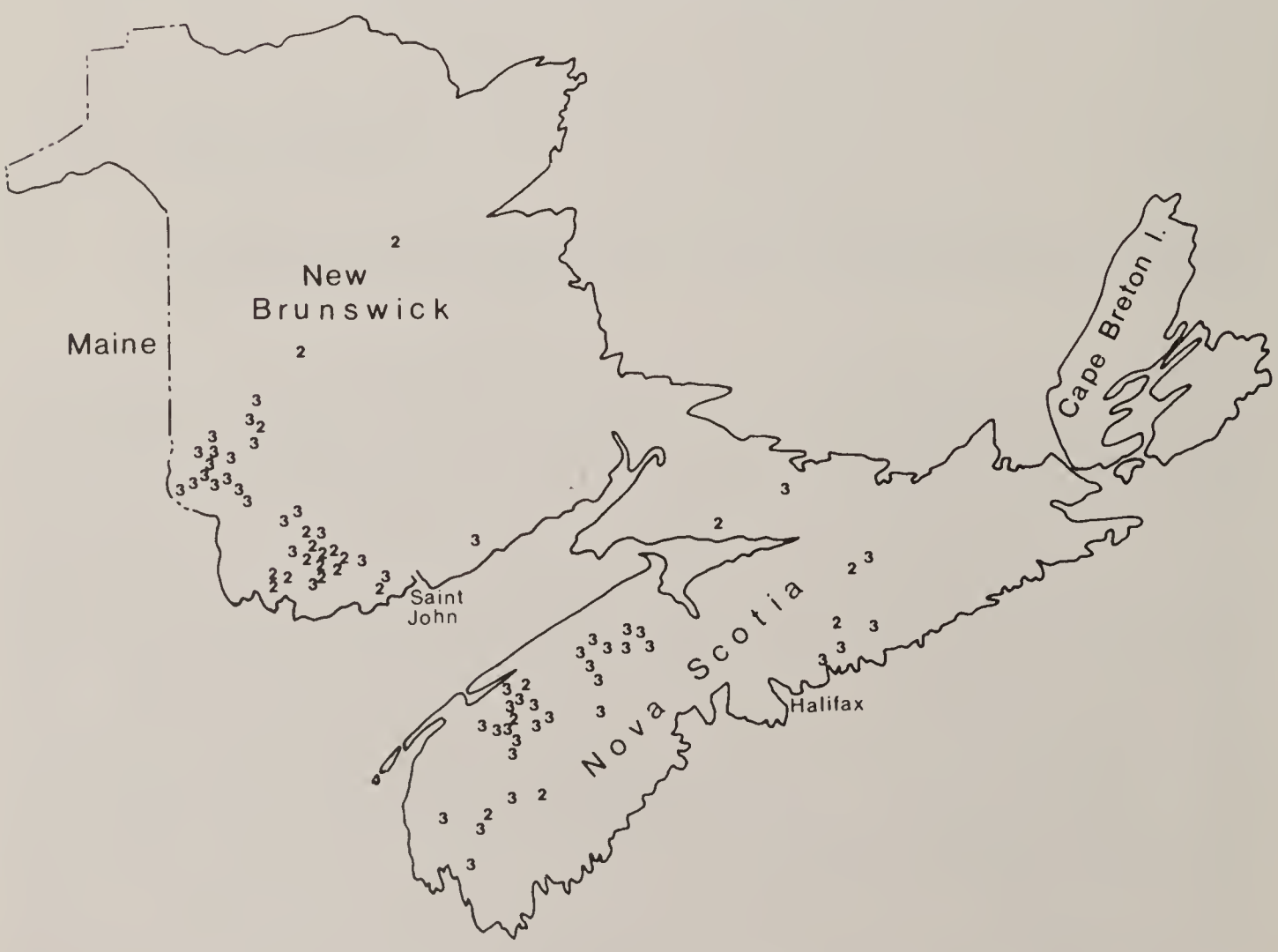

Fig 4 Geographic locations of lakes containing fish assemblages 2 and 3.

occupy the space of assemblage 5. The smallmouth bass lakes are widely separated from chain pickerel and white perch on axis 2; however, they were netted from only a small number of lakes.

Geographical location of various assemblages. Surveys were not performed uniformly throughout New Brunswick (N.B.) and Nova Scotia (N.S.). Southwestern N.B. and southwestern N.S. were surveyed most intensively, with fewer lakes surveyed in north-central N.B., eastern N.S. and Cape Breton Island (Figs 3, 4). Nevertheless, within the constraints of the surveying biases, the various species assemblages occupied different geographic locations. Assemblage 1 (brook trout only, Fig 3) was the only assemblage encountered in Cape Breton island. It was also the most numerous assemblage in north-central N.B. (8 of 10 lakes) and in the lakes sampled in southern N.B. to the east of Saint John (4 of 5 lakes). On the other hand, the assemblage was encountered only once (of 51 lakes) in southwestern Nova Scotia, and in only one of 10 lakes in eastern Nova Scotia.

Lakes with assemblage 4 (yellow perch only) were encountered only in southwestern N.S. (16 of 51 lakes) and eastern N.S. (1 of 10).

Lakes containing assemblages 2, 3 and 5 all had similar geographic distributions (again emphasizing that they are really segments of a continuum), being the predominant assemblages encountered in southwestern N.B., southwestern N.S. and eastern N.S. Lakes with assemblage 3 were most numerous: 22 of 45 lakes in southwestern N.B. 24 of 51 lakes in southwestern N.S., and 5 of 10 lakes in eastern N.S. Lakes containing assemblage 2 constituted 11 of 45 lakes in southwestern N.B., and were particularly frequent in coastal drainages to the west of Saint John (Fig 4). Assemblage 2 was in 4 of 51 lakes sampled in southwestern N.S., and 3 of 10 in eastern N.S.

Lakes with assemblage 5 were encountered least frequently - in 2 of 45 lakes in southwestern N.B., and 5 of 51 in southwestern N.S. One very acidic lake in southern N.B., from which only chain pickerel were collected, also fell into this assemblage.

Correlation of the various assemblages with lake physico-chemical parameters. When a discriminant function analysis was performed to determine significant correlations of the various fish assemblages with lake physico-chemical parameters, pH and lake area were the significant parameters in determining occurrence of the various fish assemblages (Table I). The concentration of chloride and DOC were of marginal significance. The first two discriminant functions accounted for 95% of the variance (Table II), with function 1 being primarily related to lake area and function 2 to pH. Thus assemblage 1 (brook trout only) was characteristic in lakes of less surface area than the other assemblages. The assemblages with greatest numbers of species, 3 and 5, tended to be found in lakes of greater surface area. Assemblage 4, on the other hand, was found in lakes characterized by low pH (mean of 4.6), whereas the mean pH of lakes yielding the other assemblages was over 5.6 (Table III). In Fig 1, we can envisage that axis 1 represents lakes of decreasing surface area as one moves along the axis from left to right. Brook trout tend to be found in the smallest lakes while smallmouth bass, chain pickerel and white perch tended to be found more frequently in lakes of largest surface area.

Although axis 2 is strongly influenced by pH, lakes associated with assemblages 1, 2, 3 and 5 have similar mean pH levels, and are centered at about the middle of axis 2. Only assemblage 4 is polarized at the low pH end of the axis.

Several other environmental parameters were significant in discriminant functions 3 and 4, but accounted for very little of the total variance. The significance of the concentration of chloride is probably geographical - N.B. lakes had lower mean Cl^- concentrations than N.S. lakes. Thus, assemblages 1 and 2 had a greater bias of N.B. lakes, consequently lower mean Cl^- (Table III). Dissolved organic carbon (DOC)

contributes to acidity of unbuffered waters (e.g. Kerekes *et al.*, 1986). The significance of DOC in discriminant function 4 is in part attributable to the higher mean DOC concentrations in lakes of assemblage 4. Excluding these acidic lakes, there is a trend of increasing mean DOC concentration with increasing mean lake area. The coefficient for depth was also significant in discriminant function 4 due to the shallower mean depths for lakes of assemblage 4. Lake depths recorded for this survey are based on a single sounding in each lake. More complete morphometry data on these lakes would be required to warrant further comment.

Prediction of fish assemblage from lake physico-chemical parameters. On the basis of the first two discriminant functions, assemblage membership for the various lakes was correctly predicted in 50% of the cases (Table IV). Prediction scores were highest

Table I Wilks' Lambda (U-statistic) test for significant differences among assemblage means for the 7 environmental parameters utilized in the discriminant function analysis.

Parameter	λ	F ratio (4,114 df)	Significance
$Depth^{1/2}$	0.956	1.299	0.275
pH	0.740	10.03	0.000*
Log $(K^{+}+1)$	0.976	0.705	0.590
Cl^{-}	0.934	2.029	0.095
SO_4^{2-}	0.958	1.236	0.300
log (DOC+1)	0.931	2.119	0.083
log area	0.712	11.50	0.000*

* Statistically significant.

Table II Discriminant analysis of lake fish assemblage.

Discriminant function	1	2	3	4
Percent of variance	55.5	39.1	3.2	2.0
Cumulative percent	55.5	94.6	97.8	99.8
Significance	0.000	0.001	0.833	0.696
Variables and standardized discriminant function coefficients.				
Log area	.893	-.193	.278	-.109
pH	.515	.909	-.430	.370
log (DOC+1)	.373	-.232	-.134	.128
Cl^{-}	.282	-.086	-.452	.609
log $(K^{+}+1)$	-.332	-.148	.226	.174
$Depth^{1/2}$	.101	.078	-.067	-.658
SO_4^{2-}	.015	.087	.916	.209
Pooled within-group correlations between discriminating variables and canonical discriminant functions. *: statistically significant ($p < .01$).				
Log area	.851*	-.203	.180	-.264
pH	.240	.925*	-.074	.171
SO_4^{2-}	-.48	.213	.836*	.350
$Depth^{1/2}$	.144	.263	-.057	-.650*
Cl^{-}	.130	.383	.272	.494*
log (DOC+1)	.198	-.363	-.086	.437*
log $(K^{+}+1)$	.026	.246	.062	.285*

for lakes containing assemblages 1 and 4 (62.5% and 80% correct prediction, respectively), which is reasonable since these assemblages were the most distinctive; lakes with assemblage 1 having small surface area, and those with assemblage 4 being acidic. Prediction scores for lakes with assemblages 3 and 5 were low, being no greater than could be accounted for by chance (50%). The low predictability reflects the similarity of these assemblages. If they are combined, the percentage of correct predictions for the combined lakes is 63. Thirty percent of the lakes containing assemblages 2 were predicted to contain assemblage 1, and may represent some of the smaller lakes with the brook trout-white sucker assemblage.

Table III Means of various enviornmental parameters for the fish assemblages in 119 lakes.

Assemblage	Depth (m)	Area (ha)	pH	K^+	Cl^-	SO_r^{2-} mg L^{-1}	DOC	Number
1	3.2	22.5	5.67	0.28	2.63	3.94	4.9	24
2	4.0	55.5	5.84	0.28	2.46	3.92	5.0	23
3	3.7	90.4	5.94	0.28	3.06	3.93	5.8	50
4	2.8	70.7	4.60	0.24	3.99	3.69	7.0	15
5	3.8	92.3	5.66	0.26	3.54	3.40	6.2	7

26 cases excluded from analyses.

15 had missing or out-of-range group codes (no fish caught).

11 had at least one missing discriminating variable (usually depth).

Missed species. Seventeen lakes in N.S. and 21 in N.B. included in this work have been the subject of previous surveys (Alexander *et al.*, 1986; Prov. of N.B. Dept. Natural Resources, unpubl. surveys). The species obtained in our survey are compared to those previously reported in Table V. In the first column of Table V, the percentage of 38 lakes in which each of the species considered was found is presented. For example, brook trout were sampled from 27 of 38 lakes, or 71%. Our survey, on the other hand, collected brook trout from 17 of the 38 lakes (45%, column 2). The third column of Table V shows the number of the remaining lakes in our survey (90-includes "fishless" lakes) which yielded brook trout - 51 (57%). If we assume that 71% is the percentage of all lakes surveyed which really contained brook trout; then an additional 13 of the remaining 90 lakes should also contain brook trout. Therefore, the total number of lakes which contained missed brook trout populations is estimated to be 23-10 of the 38 lakes surveyed more than once plus an estimated 13 of the 90 lakes which were only surveyed once. These estimated missed species totals are given in column 4 of Table V. The final column gives the estimated

Table IV Predicted assemblage membership based upon discriminant functions 1 and 2. Underlines show correct predictions. Overall correct percentage was 50.4%. Percentage in parentheses.

Assemblage	1	2	3	4	5	Number
1	*15(62.5)*	5(20.8)	1(4.2)	1(4.2)	2(8.3)	24(20.2)
2	7(30.4)	*10(43.5)*	2(8.7)	1(4.3)	3(13.0)	23(19.3)
3	5(10.0)	6(12.0)	*20(40.0)*	8(16.0)	11(22.0)	50(42.0)
4	1(6.7)	1(6.7)	1(6.7)	*12(80.0)*	0(0)	15(12.6)
5	1(14.3)	0(0)	2(28.6)	1(14.3)	*3(42.9)*	7(5.9)

Table V Estimation of fish species missed by our survey. Comparison of presence-absence data with data from other surveys for 38 of the 128 lakes surveyed (first two columns). Estimated numbers of lakes where a particular species may have been missed was derived by subtracting the percentage of lakes with species present in our survey (e.g. 45% for brook trout) from the percentage with species present in all surveys (71% for brook trout); and assuming that the species should have been present in the same additional percentage of the remaining 90 lakes (e.g. 26% for brook trout). Figures in parentheses are percentages.

Species	Lakes with species present, all surveys	Lakes with species present, our survey	No. of remaining lakes with species in our survey	Est. no. of lakes where species missed	Est. total no. of lakes with species (sum of previous three columns)
Salvelinus fontinalis	27 (71)	17 (45)	51 (57)	23	91
Catostomus commerconi	28 (74)	22 (58)	50 (56)	16	88
Perca flavescens	17 (45)	15 (39)	50 (56)	5	70
Ictalurus nebulosus	17 (45)	15 (39)	32 (36)	8	55
Couesius plumbeus	15 (39)	3 (8)	7 (7.8)	28	38
Notemigorus crysoleucas	8 (21)	1 (3)	7 (7.8)	16	24
Semotilus corporalis	7 (18)	5 (13)	14 (16)	4	23
Morone americanus	4 (10)	3 (8)	15 (17)	2	20
Lepomis gibbosus	4 (1)	1 (3)	6 (6.7)	6	13
Micropterus dolomieui	3 (8)	2 (5)	5 (5.6)	3	10
Esox niger	1 (3)	0 (0)	5 (5.6)	3	8
Salmo salar	3 (8)	1 (3)	0 (0)	4	5

total number of lakes (of 128) containing each species. It is apparent that some species were sampled quite reliably, for example, yellow perch were netted from 65 of the lakes, and were estimated to be missed only in an additional 5 lakes. Golden shiners and lake chub, on the other hand, were probably sampled very inefficiently. We estimate that we missed golden shiners in 16 lakes, and sampled them only from 8.

Did the missed species affect the designation of the various assemblages? Of the 38 lakes surveyed more than once, 21 were in assemblage 3 — the diverse assemblage. Additional species would not alter their classification unless possibly they were white perch or smallmouth bass (conversion to assemblage 5). As we stated above, white perch were netted very efficiently and smallmouth bass were infrequent in these headwater lakes. Therefore, it would seem that the presence of additional species would not alter many lakes assigned to assemblage 3. Six lakes surveyed more than once were lakes we found to contain brook trout only (assemblage 1). Of these 6 lakes, 3 yielded white suckers in the other surveys, which would shift them to assemblage 2. Our ordination results indicate that lakes containing assemblage 2 resembled those containing assemblage 1 in physico-chemical parameters, averaging a slightly larger surface area.

Four of the 38 lakes were found to be yellow perch-only lakes (assemblage 4) in our survey. Of these four, only one was found to contain additional species (brook trout and white perch) in other surveys. Considering the low mean pH of lakes in this category, it is probable that many of them contain no other species used in this analysis (Harvey, 1980; Smith *et al.*, 1986; Haines *et al.*, 1986).

Four of the 38 lakes yielded no fish in our survey. One of them yielded yellow perch in other surveys, indicating that it should be in lakes designated as containing assemblage 4. One other was found to contain white sucker, brook trout and lake chub, indicating that in reality it contained assemblage 2. It is probable that some of our "fishless" lakes contained either assemblages 1, 2 or 4. It seems unlikely that many of them would contain assemblages 3 or 5, as at least some of the species would have been caught.

We conclude that inclusion of missed species would not change the basic assemblages elucidated by the ordination techniques used here. The relative numbers of lakes containing the various assemblages would probably have changed - primarily due to transfer of some brook trout-only lakes (assemblage 1) to those containing white sucker and brook trout (assemblage 2).

"Fishless" lakes. Seventeen lakes in the survey yielded no fish when gill-netted. However, two of them had been found to contain fish by other surveys (see above section). In addition, one lake had contained brook trout and pumpkinseeds in a study some 40-years-ago (Smith, 1952). The percentage of lakes yielding no fish was about 8% for lakes with pH $>$4.5 (Fig 5), but rose to 33% for lakes with pH $\leq$4.5. Fishless lakes at these lowest pH levels had a larger mean lake area (Table VI), because three of the five lakes had areas of 70-150 ha. In contrast, lakes in the higher pH categories were consistently small, all with areas less than 30 ha. Although of smaller surface area the "fishless" lakes of higher pH had greater average maximum depth than the most acidic ones. The highly acidic lakes also had high average DOC.

Discussion

Five fish assemblages were identified from the ordination analysis: brook trout; brook trout-white sucker; multi-species, with white sucker, brown bullhead, and yellow perch the most frequent components; yellow perch; and another diverse assemblage with greater emphasis on white perch, chain pickerel and smallmouth bass. Lake area was the single most important variable separating these assemblages, with brook trout lakes averaging the smallest in area. With increasing lake size, the

assemblage present progressed from brook trout-white sucker, to the multi-species assemblages with assemblage 5 (emphasizing white perch and smallmouth bass) inhabiting the lakes of greatest area.

The trend of increasing species richness with increasing lake area has been observed previously (Johnson *et al.*, 1977; Eadie and Keast, 1984; Rahel, 1984; Haines *et al.*, 1986; Kelso *et al.*, 1986; Matuszek and Beggs, 1988). The explanation may lie in the immigration-extinction theory of MacArthur and Wilson (1967) or in the fact that lakes with more surface area are liable to have a greater diversity of habitats, and can thus support more diverse fish populations. The fact that the group of lakes with least surface area (brook trout lakes) are no shallower than the other lake groups may be indicative of lesser littoral areas in these lakes, a factor in limiting species diversity.

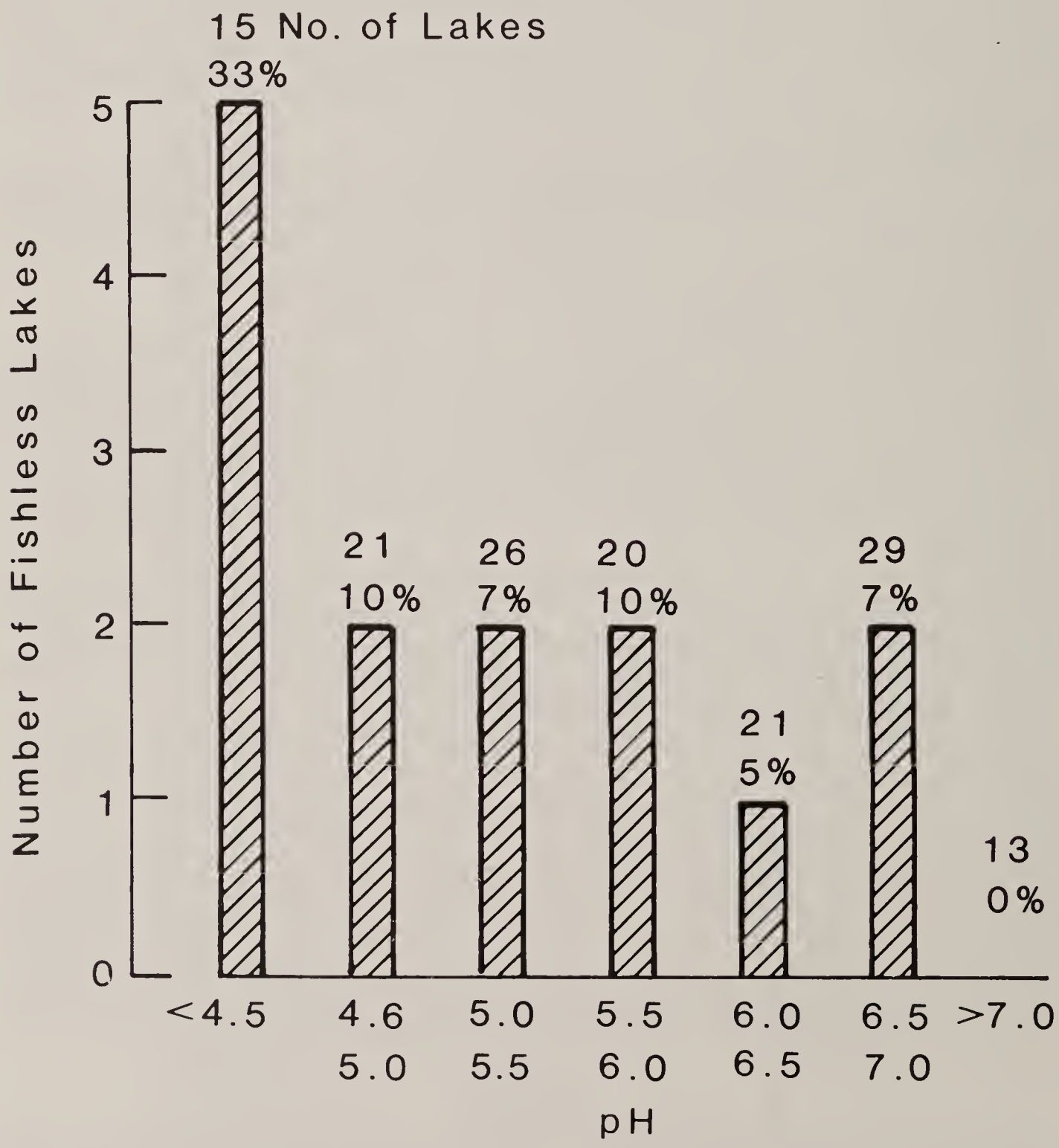

Fig 5 Numbers of sampled lakes which yielded no fish for seven pH categories. Upper number above the bar is the total number of lakes in the category. The lower number is the percentage of the lakes in the category which yielded no fish.

Table VI Means of physico-chemical parameters of lakes yielding no fish.

pH class of lakes	Area (ha)	Depth (m)	K^+	Cl^-	pH	SO_4^{2-} mg L^{-1}	DOC	Number
$\leq$ 4.5	73	2.5	.24	3.5	4.43	4.1	9.7	5
4.6 < 5.5	20	4.3	.20	3.0	5.01	3.4	3.4	4
> 5.5	21	4.9	.20	2.4	6.29	4.1	4.7	5

Haines et *al.* (1986) found their "depauperate" lakes to be in this category. Brook trout may not require extensive littoral areas for successful reproduction or may be superior competitors in homogenous habitats. Alexander et *al.* (1986) also found brook trout presence to be correlated negatively with most other species - probably due to the brook trout-only lakes in their data base. Many of the brook trout lakes, however, probably contained the ninespine stickleback as well, a species too small to be caught in our gill-nets.

Lake area is a factor determining the morphoedaphic index (*sensu* Ryder, 1965) of lakes, which is correlated with lake productivity. Consequently, it may be inferred -other factors being equal - that the brook trout lakes are probably the least productive of lakes. This concept is consistent with the usual eutrophication sequence where the salmonid community of low diversity is supplemented by percid, then cyprinid communities as eutrophication proceeds. Higher morphoedaphic indices have been correlated with high rates of eutrophication (Kelso et *al.*, 1986).

The white sucker-brook trout assemblage (assemblage 2) inhabits lakes of slightly greater mean area than the brook trout lakes. Otherwise, the other physico-chemical parameters are very similar for these two lake types. Although insufficient temperature records for the lakes were available to permit its inclusion as a parameter in the discriminant function analysis, lakes containing assemblages 1 and 2 may have cooler temperature regimes. Brook trout and lake chub, other members of assemblage 2, are considered "cool water" species (e.g. Greeley, 1931).

The diverse species assemblage (assemblage 3) occupies headwater lakes of greater area and forms a continuum with assemblage 5 in the larger lakes. Study of larger numbers of lakes with more thorough sampling might reveal patterns within this assemblage which cannot be seen with the present data. This assemblage is probably also common in higher order lakes of the Maritime region. The "centrachid" and "cyprinid" assemblages of Haines et *al.* (1986) were not distinguishable in our lake data. Assemblage 3 might have broken down further had we used additional sampling methods which collected small species efficiently.

The yellow perch-only lakes are similar in physico-chemical characteristics to those containing assemblage 3, but are more acidic and tend to have higher levels of DOC. Higher DOC is often correlated with a lower pH in poorly buffered waters (Kerekes et *al.*, 1986). Consequently, these lakes are probably receiving more organic acidity than lakes in the other assemblages. In addition to natural organic acidity, there is evidence that pH reduction in waters of low alkalinity occurs through atmospheric transport of acidity to these waters (Kerekes et *al.*, 1986) - particularly in seasons of high discharge. Fish populations in lakes which are initially naturally more acidic would seem to be at greater risk from anthropogenic acidification. Smith et *al.* (1986) found that the minimum pH tolerated by most species was 4.7-4.8, with the yellow perch tolerating pH levels to 4.4. Rahel (1984) found a yellow perch-*Umbra* assemblage to be associated with the acid Wisconsin lakes. *Umbra* does not occur in the Maritime provinces, nor does it attain a size to allow it to be caught by standard gill-nets. Rahel (1984) also found a "cyprinid" assemblage indicative of lakes with low

oxygen partial pressures under ice cover. Some of the "fishless" lakes of N.B. may be so due to low winter oxygen values.

Lakes containing assemblage 5 were of greatest surface area, and may not be qualitatively different in reality from those with assemblage 3. Some of these lakes, were not headwater lakes. Categorizing lakes on the basis of smallmouth bass presence-absence is questionable, as its distribution is determined mainly through recent introductions by humans.

In summary, three factors - lake area, lake acidity, and geographic locality appear primarily responsible for determining species composition of Maritime lakes. High lake acidity seems to be correlated with low species richness and presence of a yellow perch assemblage. Lakes of small surface area are frequently inhabited only by brook trout. This study suggests that 10-15% of the headwater lakes surveyed are too acidic for the survival of any potential sport fish species other than yellow perch. Most of these lakes occur in southwestern N.S. Watt *et al.* (1983) have made a case for lowered pH in rivers of southwestern N.S. over the last 30-40 years. This conclusion was based primarily upon the former presence of salmon runs in rivers which are now too acidic to permit salmon survival. The conclusion is supported by some (admittedly scanty) pH measurements of 30 years ago. If rivers of this area are now more acidic, it would seem reasonable to suppose that lakes of the area are more acidic as well, since lakes and rivers form linked drainage systems. If this hypothesis is correct, then populations of brook trout, white suckers, brown bullhead and several other species frequently associated with assemblage 3 may well have been lost. The data presented here indicate that 20-25% of headwater lakes in southwestern N.S. are too acidic for larger species other than yellow perch.

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References

Alexander, D.R., Kerekes, J.J. and **Sabean, B.C.** 1986. Description of selected lake characteristics and occurrence of fish species in 781 Nova Scotia lakes. *Proc. N.S. Inst. Sci.* 36: 63-106.

Beamish, R.J. and **Harvey, H.H.** 1972. Acidification of the La Cloche Mountain lakes, Ontario, and resulting fish mortalities. *J. Fish. Res. Board Can.* 29: 1131-1143.

Colby, P.J., Spangler, G.R., Hurley, D.A. and **McCombie, A.W.** 1972. Effects of eutrophication on salmonid communities in oligotrophic lakes. *J. Fish. Res. Board Can.* 29: 975-983.

Eadie, J. McA., Hurley, T.A., Montgomery, R.D. and **Teather, T.L.** 1986. Lakes and rivers as islands: species-area relationships in the fish faunas of Ontario. *Environ. Biol. Fishes* 15 (12): 81-89.

Eadie, S. and **Keast, A.** 1984. Resource heterogeneity and fish species diversity in lakes. *Can. J. Zool.* 62: 1689-1695.

Gauch, H.G. Jr. 1982. Multivariate analysis in community ecology. Cambridge Univ. Press, 298 p.

Greeley, J.R. 1931. Fishes of the area, with annotated list. *In* A biological survey of the St. Lawrence Watershed. New York Cons. Dept. Biological Survey No. 5: 44-94.

Green, R.H. and **Vascotto, G.L.** 1978. A method for the analysis of environmental factors controlling patterns of species composition in aquatic communities. *Water Res.* 12: 583-590.

Haines, T.A., Pauwels, S.J. and **Jagoe, C.H.** 1986. Predicting and evaluating the effects of acidic precipitation on water chemistry and endemic fish populations in the northeastern United States. Fish and Wildlife Service, U.S. Dept. Int. Biol. Rep. 80 (40.23) 139 p.

Hill, H. 1980. Widespread and diverse changes in the biota of North American lakes and rivers coincident with acidification, p. 93-98. *In* D. Drablos and A. Tollan (ed.) Ecological impact of acid precipitation. Proc. Int. Conf., Sandefjord, Norway.

Hill, M.O. 1979a. DECORANA - A FORTRAN program for detrended correspondence analysis and reciprocal averaging. Ecol. and Systematics, Cornell Univ., Ithaca, N.Y.

Hill, M.O. 1979b. TWINSPAN - A FORTRAN program for arranging multivariate data in an ordered two-way table by classification of the individuals and attributes. Ecol. and Systematics, Cornell Univ., Ithaca, N.Y.

Johnson, M.G., Leach, J.H., Minns, C.K. and **Oliver, C.H.** 1977. Limnological characteristics of Ontario lakes in relation to associations of walleye (*Stizostedion vitreum* northern pike (*Esoc lucius*), lake trout (*Salvelinus namaycush*), and smallmouth bass (*Micropterus dolomieui*). *J. Fish. Res. Board Can.* 34: 1592-1601.

Kelso, S.R.M., Minns, C.K., Gray, J.E. and **Jones, M.L.** 1986. Acidification of surface waters in eastern Canada and its relationship to aquatic biota. Can. Spec. Publ. Fish. Aquat. Sci. 87: 42 p.

Kerekes, J., Beauchamp, S. Tordon, R., Tremblay, C. and **Pollock, T.** 1986. Organic versus anthropogeni acidity in tributaries of the Kejimkujik watersheds in western Nova Scotia. *Wat. Air Soil Poll'n.* 31: 165-173.

Leach, J.N., Johnson, M.G., Kelso, J.R.M., Hartman, J., Numann, W. and **Entz, B.** 1977. Responses of percid fishes and their habitats to eutrophication. *J. Fish. Res. Board Can. 34:* 1963-1971.

Matuszek, J.E. and **Beggs, G.L.** 1988. Fish species richness in relation to lake area, pH and other abiotic factors in Ontario lakes. *Can. J. Fish. Aquat. Sci.* 45: 1931-1941.

Muniz, I.P., Sevaldrad, I.H. and **Lindheim, A.** 1976. Acid lakes and inland fisheries in south Norway. Preliminary results from an interview survey in autumn 1974. SNSF-project TN 21/76, 41 p.

Peterson, R.H., Townsend, D. and **Martin-Robichaud, D.J.** 1986. Water chemistry of 145 New Brunswick and Nova Scotia headwater lakes. Can. Tech. Rep. Fish. Aquat. Sci. 1493: iii = 36 p.

Rahel, F.J. 1984. Factors structuring fish assemblages along a bog lake successional gradient. *Ecology* 65: 1276-1289.

Ryder, R.A. 1965. A method for estimating the potential fish production of north-temperate lakes. *Trans. Am. Fish. Soc.* 94: 214-218.

Schofield, C.L. 1976. Acid precipitation: effects on fish. *Ambio* 5: 228-230.

Smith, M.W. 1952. Limnology and trout angling in Charlotte County lakes, New Brunswick. *J. Fish. Res. Board Can.* 8: 383-452.

Smith, D.L., Underwood, J.K., Ogden, J.G. and **Sabean, B.C.** 1986. Fish species distribution and water chemistry in Nova Scotia lakes. Water Air Soil Pollut. 30: 489-496.

Snekvik, E. 1974. Fish populations in acidified water-courses in the southernmost part of the country. Directoratet for jakt og ferskvannsfisk, Âs. Stensilert rapport, 47 p.

SPSS[x] User's Guide, 2nd Ed., SPSS Inc., 1986.

van Leuven, R.S.E.W. and **Oyen,** F.G.F. 1987. Impact of acidification and eutrophication on the distribution of fish species in shallow and lentic soft waters of The Netherlands: an historical perspective. *J. Fish. Biol.* 31: 753-774.

Watt, W.D., Scott, C.D. and **White, W.J.** 1983. Evidence of acidification of some Nova Scotian rivers and its impact on Atlantic salmon *Salmo salar.* Can. J. Fish Aquat. Sci. 40: 462-473.

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Appendix 1 The lakes surveyed are shown in sequence as ordered along axis 1 of the detrended correspondence analysis (accounting for 48% of the variance). Various species used in the analysis are arrayed horizontally. Lake numbers listed in column at the far right matched those in Peterson et al. (1986) so that fish species can be matched to water chemistry if desired. Numbers prefaced with a 1 are New Brunswick lakes (e.g. 1-66), those with a 2 are Nova Scotia lakes. The letters in the column second from the right represent results of a hierarchial cluster analysis of the fish species data (Twinspan; Hill, 1979b). Lakes with the same letter were clustered together. Horizontal dotted lines indicate designated assemblages. Legend for the fish species abbreviations are: BT (brook trout), LC (lake chub), LLS (landlocked salmon), WS (white sucker), BB (brown bullhead), FF (fallfish), PS (pumpkinseed), GS (golden shiner), YP (yellow perch), WP (white perch), CP (chain pickerel), SmB (smallmouth bass). Numbers for the various species are relative abundances, as explained in the text.

Fish species													Lake
BT	LC	LLS	WS	BB	FF	PS	GS	YP	WP	CP	SmB	Cluster	no.
Assemblage 1													
2												A	1-66
1												A	2-42
1												A	1-7
2												A	1-54
2												A	1-51
2												A	1-25
2												A	1-24
1												A	1-6
2												A	1-10
4												A	2-75
4												A	2-74
4												A	2-73
3												A	2-76
4												A	2-72
3												A	1-69
2												A	1-1
3												A	1-67
3												A	1-65
1												A	1-4
4												A	2-71
4												A	1-63
4												A	2-57
4												A	1-59
4												A	1-58
4												A	1-57
4												A	1-60
Assemblage 2													
2			2									A	1-27
3			3									A	1-33
1			1									B	1-16
1	1			1								C	1-18
4			3	2								C	1-21
2			2					1				B	2-3
1			1									B	1-15
1			1									B	1-2
2			1	1				1				C	2-6
1			2									B	1-26
1			3									B	1-13

Appendix 1 (cont'd)

Fish species													Lake
BT	LC	LLS	WS	BB	FF	PS	GS	YP	WP	CP	SmB	Cluster	no.
3			4		1							B	1-12
1			4									B	2-66
1			1	1	1							B	1-56
1				2								C	1-17
1			3									B	2-61
1			3									B	2-59
1			2	1								B	2-62
1	3		4					1				C	1-62
1			4									B	1-29
Assemblage 3													
1			2					1				C	1-55
1			2		1		1	1				C	1-53
2	1	1	3	2	1							C	1-45
			2	1				1				C	2-39
1			2	1								B	1-14
1			2					1				C	2-11
			4	2								C	1-40
			1	2				1				C	2-30
1			2		1			2				C	1-31
			3	2				1				C	2-2
		1	2		1		2	1				C	1-19
			1	1								C	2-5
			2					1				D	1-3
			2	1				1				C	2-48
1			2						1			B	2-63
			3	2	1	1						C	1-46
1			3	2				2				C	2-10
1			2	1	2	1		2				C	1-48
1			4	2				3				C	2-60
			2	2				1				C	2-7
	1		1	2								C	2-40
1								1				E	2-35
1								1				E	2-36
			4	1	2	2	1	4				C	1-34
	1		1	1	3			1				C	1-50
			4	2	2	1		3			1	C	1-36
			3	1	2	1	1	2				C	1-37
					1							F	1-68
					1							F	1-5
			1	1		1		1				C	1-22
	1		3									C	1-30
				4					1			C	2-56
			2					1	2			G	1-32
			2	1				2				C	2-8
	1		1	2				2				C	2-9
			2	2			1	2				C	2-22

Appendix 1 (cont'd)

Fish species												Cluster	Lake no.
BT	LC	LLS	WS	BB	FF	PS	GS	YP	WP	CP	SmB		
			3	1				4				C	2-51
1				3				1	2			C	2-33
			2	2			1	2				C	2-47
1								1				E	2-32
			3						3			G	1-43
			1	3				3				C	2-38
			1					1				D	2-49
			3	2			3	4				C	2-58
			2	1	1				1	2		G	1-44
			3	1	1			1	3	2		G	1-39
	1		1						1		1	G	1-47
1								2				B	2-65
			1		1			1		1	1	D	1-38
			2	1	1	1	1	1	3	1		G	1-42
			2	2				4				C	2-50
			4	1				1	4			G	2-4
			1	1				4				C	2-43
			1					2				D	2-15
			1					2				D	2-13
			2	2	1		1		4			G	2-23
Assemblage 4													
								1				H	2-68
								3				D	2-14
								1				H	2-54
								2				D	2-27
								1				H	2-53
								1				H	2-45
								2				D	2-25
								2				D	2-19
								1				H	2-44
								1				H	2-37
								1				H	2-31
								1				H	2-29
								1				H	2-28
								1				H	2-24
								1				H	2-21
								1				H	2-17
								1				H	2-12
Assemblage 5													
			2	2				2	4			C	2-52
1			3	1				1	4			G	2-41
			1	1				2	3			G	2-55
			2	2					4		2	G	1-41
								4			3	D	1-49
1			1	1				1	4			G	2-34
										2		F	1-64
			1					1			4	D	2-46

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AN EXAMINATION OF NORTH AND SOUTH AMERICAN ISOLATES OF PITHOMYCES CHARTARUM FOR PRODUCTION OF SPORIDESMIN AND SPORIDESMOLIDES[1]

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Two hundred and fifty two isolates of *Pithomyces chartarum* have been collected from pastures in Brazil (101), Colombia (30), Texas (5), and Nova Scotia (116) during the last 20 years. It has been possible to examine 57 of these, 2 from Texas, 3 from Nova Scotia, 20 from Colombia, and 32 from Brazil, in detail. Twenty-one of these isolates produced spores on rye-grain medium and all produced sporidesmolides I to V in similar proportions. Ten of the isolates also produced metabolites that inhibited the growth of *Micrococcus luteus* but none of the isolates produced sporidesmin or any of its known 9 congeners in amount greater than the detectable limits. Thus *P. chartarum* in these American countries appear to differ physiologically from isolates of the same species found in Australia, New Zealand, and South Africa.

Deux cent cinquante-deux échantillons isolés de *Pithomyces chartarum* ont été collectionés de pâturages du Brésil (101), de la Colombia (30), du Texas (5), et de la Nouvelle-Écosse pendant les 20 dernières années. On a pu examiner en détail 57 de ceux-ci, 2 du Texas, 3 de la Nouvelle-Écosse, 20 de la Colombie, et 32 du Brésil. Vingt-et-un de ces isolats ont produit des spores sur du milieu au seigle, et tous ont produit des sporidesmolides I à V en proportions egales. Des métabolites de dix isolats ont inhibé la croissance de *Micrococcus luteus,* mais nul isolat a produit du sporidesmin ou aucun de ses 9 congénères connus, en montant susceptible au repérage. *P. chartarum* des pays américains nommés ci-dessus semble alors se différencier du point physiologique des isolats de la même espèce découverts en Australie, en Nouvelle Zelande, et en Afrique du Sud.

Introduction

Photosensitivity is a common symptom of diseases of sheep and cattle that graze permanent pasture in the sub-tropics. It has been reported in Argentina (Regalado, 1981), Australia (Flynn, 1962), Brazil (Döbereiner *et al.*, 1976), Colombia (Aycardi *et al.*, 1982), New Zealand (Done *et al.*, 1960), South Africa (Kellerman *et al.*, Marasas *et al.*, 1972) and Texas (Halder *et al.*, 1981; Taber *et al.*, 1968). In all cases, the occurrence of photosensitivity was associated with dense populations of *Pithomyces chartarum* on the herbage debris. Isolates of this fungus from such areas in New Zealand, Australia and South Africa have been shown to produce a group of about 9 related metabolites known as sporidesmins (Fig. 1; Leigh and Taylor, 1976). Extensive toxicity studies of one of these metabolites have enabled veterinarians to reproduce many of the

[1]NRCC No. 30325.

features of photosensitivity observed in the field (Taylor, 1971). However it has not been possible to demonstrate the production of sporidesmins by isolates of *P. chartarum* collected from pastures in Texas where cattle were photosensitive (Halder *et al.*, 1981; Ueno *et al.*, 1974).

During the last 25 years workers in several places in North and South America have sent to the Atlantic Research Laboratory (ARL), National Research Council of Canada, isolates of *P. chartarum* that they had obtained from pastures where sheep and cattle had been photosensitive. In many cases these workers had been unable to demonstrate the production of sporidesmin by these cultures. It seemed possible that an explanation of the difference in results in the Americas from those, e.g. in New Zealand might be due to misidentification because of the great morphological similarity between, e.g. *P. chartarum* and *P. maydicus* (Dingley, 1962) and/or that the American isolates produced a different toxin(s). We have therefore examined 54 of the 139 isolates sent to ARL in detail and have also subjected 3 isolates from the ARL Nova Scotian collection of 116, one of which had shown biological activity in a preliminary screen (Brewer *et al.*, 1974) to the same detailed investigation. The results of this work are reported.

Materials and Methods

Organisms. Pithomyces chartarum (Berk. & Curt.) M.B. Ellis, HLX 133 was obtained in New Zealand from a high-sporing sector of a wild isolate (Dingley *et al.*, 1962). Isolates HLX 975 and 976 were obtained from Dr. W.A. Taber, Texas A&M University, College Station, Texas. Isolates HLX 1533 to HLX 1581 and HLX 1605 to 1607 were from Brazil. The isolates HLX 1582 to 1604 were from Colombia. Isolates HLX 1159, 1160 and 1635 were collected with a spore-trap at Nappan, Nova Scotia. *Micrococcus luteus* HLX 701 was maintained, cultivated and used as an assay organism as previously described (Liss *et al.*, 1985).

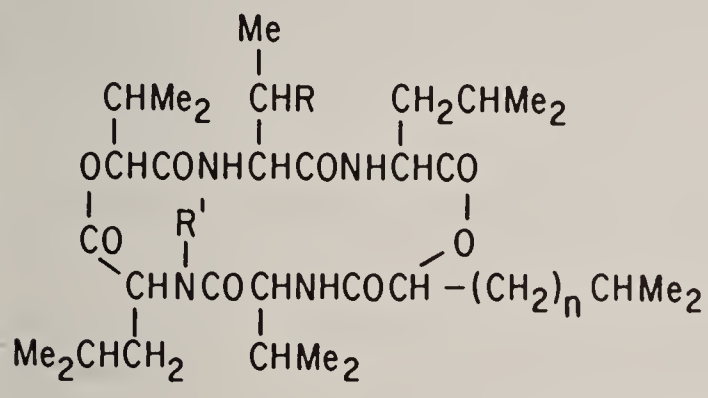
Cultivation of Organisms. All of the isolates were grown on rye grain (*Secale cereale*, Done *et al.*, 1961). Those isolates that sporulated feebly on rye (HLX 1159, 1160, 1557, 1574 and 1635) were also cultivated on the following medium (g L^{-1}): agar (Difco), 20; maltose, 30; Casitone (Difco), 3.5; yeast extract (Difco), 1; KH_2PO_4, 1; KCl, 0.5; $MgSO_4 7H_2O$, 0.5; $FeSO_4 x H_2O$, 0.01; $CaCl_2 2H_20$, 0.037; and trace metal solution (Ross, 1960) 10 mL. All fermentations were carried out on lots of 4 or 5 isolates and in addition, as a positive control, a fermentation of HLX 133 on the same scale was included.

Harvesting and Extraction. The rye-grain cultures (usually 10) were mixed with 80% (v/v) methyl alcohol-water (ca 200 mL), were combined and the mixture was stirred for 24 h, was filtered and the filtrate evaporated. The residue was taken up in methyl alcochol (300mL), petroleum ether (b.p. 30-60°C, 300 mL) and water (15 mL) were added, the phases separated (some required centrifugation at 1500 r min^{-1}) and the lower phase was re-extracted (x 3) with petroleum ether (300 mL). The methanolic phase was evaporated the residue treated with water (50 mL), and the mixture either extracted continuously for 18 h with diethyl ether or, in about half of the fermentations, extracted with ether (x 3) in a tap funnel. The ethereal extract was evaporated and the residue used for biological assay and chemical analysis.

Isolation of Sporidesmolides (Fig 1). The residue from the rye-grain cultures was suspended in chloroform (2 L), the mixture filtered and the residue washed twice with chloroform. The cultures on agar were treated similarly, but with smaller, appropriate volumes of chloroform. The extract and washings were combined and evaporated and the residue was triturated with diethyl ether. The precipitated solid was purified to give the total sporidesmolide fraction as described previously (Russell *et al.*, 1962).

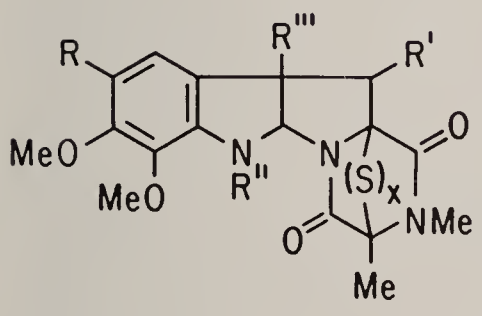
Analysis. Thin layer chromatography (t.l.c.) was carried out on Merck SiO_2 plates (20 x 20 x 0.01 cm) and benzene-ethyl acetate (4:1) was used as the developing solvent. Metabolites were detected on the plates by their ultraviolet absorption and by spraying the plates with neutral aqueous silver nitrate solution (1%, w/v) (Rahman *et al.*, 1970). Extracts were dissolved in methyl alcohol (*ca* 10 mg mL^{-1}) and two samples (0.5 mL) taken, both treated with 0.01*N* iodine solution (5 mL) and one with sodium azide (1 mL, 1% w/v). The 2 solutions were titrated with standard sodium arsenite and the concentrations of disulphides calculated as described (Brewer and Taylor, 1967). Similar solutions (0.05 mL) of the extracts were used for the disc bioassay. All bioassays were duplicated and all incorporated sporidesmin as a positive control.

Sporidesmolides (Fig 1) were analysed by high pressure liquid chromatography (h.p.l.c.). A Zorbax ODS column (DuPont, 25 x 0.46 cm) was used. The mobile phase was methyl alcohol-water (41:9), the flow rate 0.7 mL min^{-1} and the

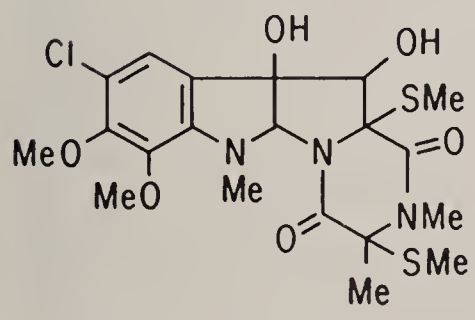


Sporidesmolides

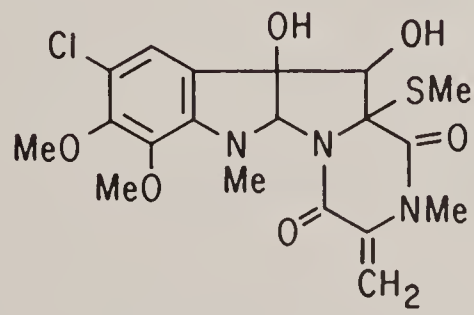
I R = R' = Me, n = 0
II R = Et, R' = Me, n = 0
III R' = H, R = Me, n = 0
IV R = R' = Me, n = 1
V R = Et, R' = Me, n = 1



Sporidesmin	R = Cl, R' = R''' = OH, R'' = Me, x = 2
B	R = Cl, R''' = OH, R' = H, R'' = Me, x = 2
E	R = Cl, R' = R''' = OH, R'' = Me, x = 3
G	R = Cl, R' = R''' = OH, R'' = Me, x = 4
H	R = H, R''' = Cl, R' = H, R'' = Me, x = 2
J	R = Cl, R' = R''' = OH, R'' = H, x = 2



Sporidesmin D



Sporidesmin F

Fig 1 Chemical formulae of sporidesmolides and sporidesmins.

separation was conducted at 50°. Sporidesmolides (2-20 mg mL^{-1}) in chloroform (or dichloromethane)-ethyl alcohol (3:1) were injected onto the column using a Waters injection block and detected by refractometry (Waters 410 refractometer). Calculation of the percentage composition of the eluted sporidesmolides was done by a Waters data module (Model 730).

Results

Experimentally, the production of a metabolite by a fungus can only be demonstrated when the metabolite accummulates in the culture medium in a concentration greater than the detection limit of a chemical or biological assay. In the case of sporidesmin(s) (Fig 1) the most convenient biological assay, to our knowledge, is that using *M. luteus* as the assay organism. In Fig 2 the relationship of zones of inhibition of this organism with respect to concentration of sporidesmin is plotted and it can be seen from this relationship that the minimum quantity of sporidesmin detectable is 1.5 μg.

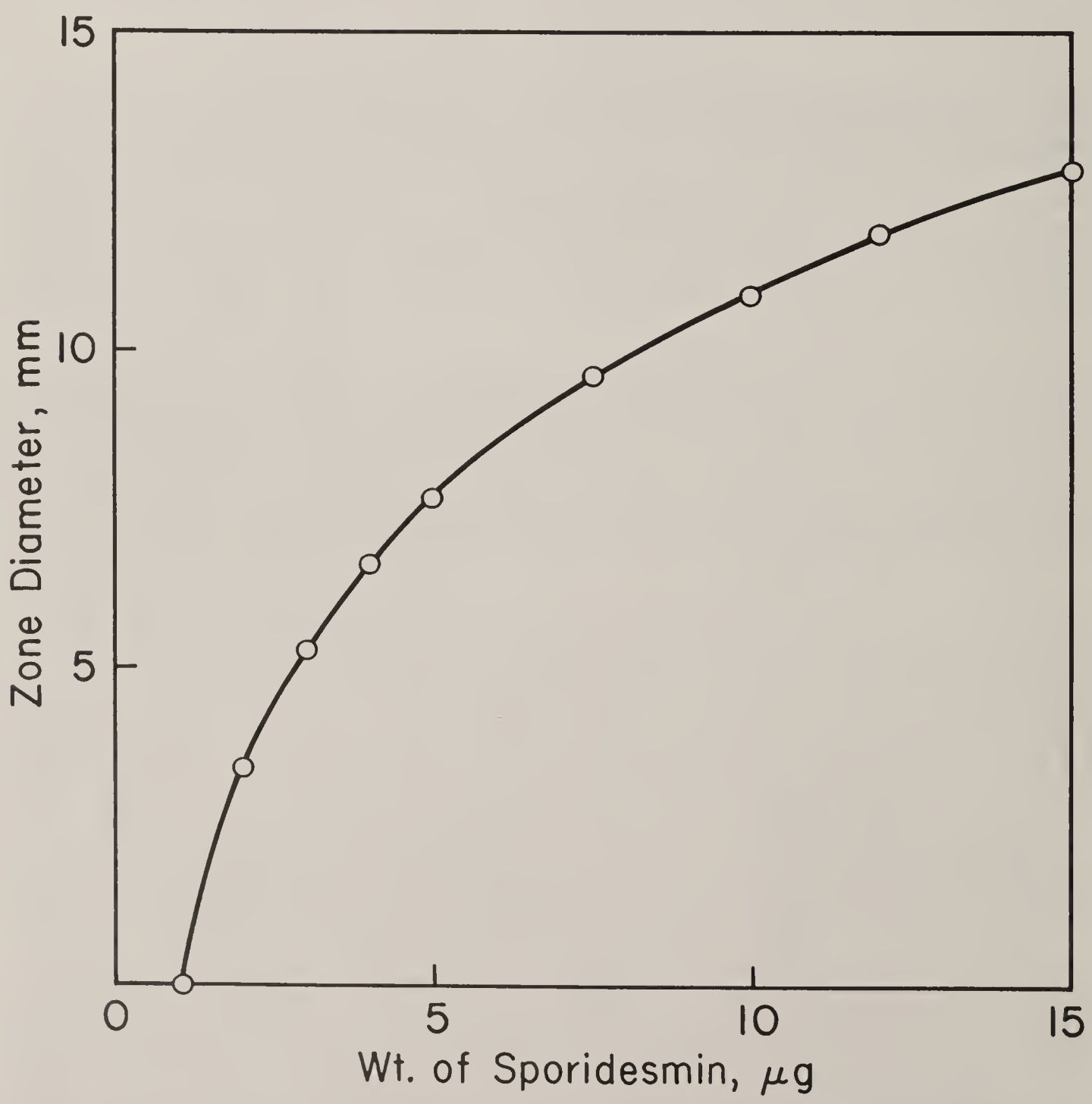


Fig 2 Growth inhibitory activity of sporidesmin against *Micrococcus luteus*.

In the case of chemical analyses of sporidesmin, 0.1 - 0.5 μg can be detected on thin layer chromatograms by reflectance ultraviolet light or by spraying the plates with neutral aqueous silver nitrate. Thus, if 750 μg of an extract suspected of containing sporidesmin is chromatographed, sporidesmin present in about 0.03% can be detected. Volumetrically, (Brewer and Taylor, 1967) sporidesmin concentrations of about 0.01% can be reliably estimated.

The weights of extracts grown on 750 g of rye grain lay in the range 0.3 - 1.2 g. A maximum of 5 mg of this material could be analysed by any of the above methods before materials other than sporidesmin interfered with the analysis. Thus 90-360 μg of sporidesmin in 750 g of nutrients could be detected.

The results reported here are in no way a thorough survey of *Pithomyces* in different countries, but rather an examination of different isolates that were available. In all cases where no activity was observed, this result is indicated in Table I by a blank.

The isolates were grown in groups of 4 or 5 and each group included an HLX 133 fermentation on the same scale. Sporidesmin was produced in all 12 HLX 133 fermentations, the yield from 10 flasks of the rye-grain medium being 226 ± 61 mg, and 40 μg of the extracts gave inhibition zone diameters in the range 9-12 mm. By comparison the biological activities given in Table I are low, with the possible exception of HLX 1604. The extract from this isolate contained a component(s) that has the same R_F as sporidesmin in the chromatographic system used, but the material did not react with silver nitrate.

In only 6 cases (HLX 1533, 1537, 1557, 1569, 1582, and 1598) were extracts obtained that were biologically active and also catalysed the decomposition of azide in the presence of iodine. Chromatography of these extracts showed that no sporidesmin or known sporidesmin derivative was present in concentrations above the minimum detectable limit. In 2 cases, HLX 1533 and HLX 1537, spots on chromatograms were obtained that reacted with silver nitrate, but their R_F corresponded to no known sporidesmin congener.

These results suggested to us that the organisms isolated in North and South America might belong to a different species of *Pithomyces*. *Pithomyces maydicus* (IMI 46232, 98084) is morphologically very similar to *P. chartarum* (Dingley, 1962) and the two fungi are most easily distinguished by the different sporidesmolides found in the spicules of their spore coats (Bertraud *et al.*, 1963; Bishop *et al.*, 1965). Recently it has been shown* that the sporidesmolides found on the spores of HLX 133 are a more complex mixture than hitherto supposed (Taylor, 1970). This increased complexity, however, probably serves to characterise the spicules on a particular isolate more rigorously. The isolates examined, that produced spores prolifically, were therefore analysed for the composition of the sporidesmolides in their spicular coats. These organisms (21 of 57) are indicated in Table I by the appearance of analytical results in the sporidesmolide column. Sporidesmolides were isolated and purified from 5 (HLX 1548, 1585, 1590, 1598 and 1607) of these 21 cultures. The melting points of these samples varied in the range 246-251° and their specific optical rotations ($[\alpha]_D^{24}$, c 0.5 $CHCl_3$) lay in the range -200° to -207°. All gave leucine, allo-isoleucine, N-methyl-leucine and valine after acid hydrolysis (Russell, 1962). The mean value for the sporidesmolide I (Fig 1) component of the mixture in the 21 samples was $63 \pm 6\%$. It may be concluded that none of these isolates can be classified as *P. maydicus* and that their identification as *P. chartarum* is correct.

*It is intended to publish full details of the hitherto undescribed metabolites of *P. chartarum* in the Canadian Journal of Chemistry as soon as possible.

Table I Geographical origin, plant substrates and production of sporidesmolides and toxic metabolites by isolates of *Pithomyces chartarum*.

Accession No. (HLX)	Origin	Substrate	Yield	Sporidesmolides % Composition					Bioassay (HLX 701)		
				I	II	III	IV	V	Wt. on disc mg	Inhibition Zone diameter mm	$AgNO_3$ + N_3^-/I_2^{**}
	U.S.A										
975	Texas	Herbage	23	73	15	3	7	2	4	10	
976	Texas	Herbage							3	1	
	Canada										
1159	Nova Scotia	Herbage	83*	69	28	1	+	1		8	
1160	Nova Scotia	Herbage	43*	62	35	1	+	1	7	14	
	Brazil										
1533	Sao Paulo	*Linum usitatissimum*							1	8	+
1535	Mina Gerais	*Pennisetum purpureum*							1	3	
1537	Sao Paulo	*Paspalum notatum*							1	5	+
1540	Sao Paulo	*Oryza sativa*									
1542	Mato Grasso	*Oryza sativa*									
1544	Sao Paulo	*Oryza sativa*									
1545	Sao Paulo	*Brachiaria mutica*	19	59	21	7	8	4			
1548	Sao Paulo	*Brachiaria decumbens*	63	64	24	2	7	3			
1549	Sao Paulo	*Brachiaria decumbens*									
1550	Sao Paulo	*Panicum maximum*									+
1551	Sao Paulo	*Pennisetum purpureum*									
1553	Sao Paulo	*Oryza sativa*									
1554	Sao Paulo	*Oryza sativa*	20	69	18	2	9	2			
1556	Sao Paulo	*Oryza sativa*									
1557	Sao Paulo	*Panicum maximum*	59*	54	39	2	+	4	1	3	+
1558	Sao Paulo	*Oryza sativa*									
1565	Rio de Janeiro	*Brachiaria decumbens*									
1566	Sao Paulo	*Brachiaria decumbens*							1.5	4	
1567	Goias	*Panicum maximum*									
1568	Goias	*Pennisetum purpureum*									
1569	Goias	*Brachiaria decumbens*							1	3	+
1571	Sao Paulo	*Brachiaria decumbens*	20	63	23	2	10	3			
1573	Brasilia	*Desmodium intortum*	29	68	22	2	5	2			
1574	Sao Paulo	*Paspalum plicatulum*	155*	60	35	3	+	2			

1577	Sao Paulo	*Stylosanthes sp.*							1	3	
1578	Goias	*Panicum maximum*	55	51	34	4	7	4			+
1579	Sao Paulo	*Panicum maximum*									
1580	Sao Paulo	*Setaria anceps*									
1581	Sao Paulo	*Oryza sativa*									
	Colombia										
1582	Santander	*Brachiaria sp.*							6	10	+
1583	Santander	*Brachiaria sp.*									+
1584	Limonar	*Panicum sp.*									
1585	Santander	*Andropogon sp.*	93	72	16	1	7	4			
1586	Santander	*Andropogon gayanus*	11	64	18	2	12	3			
1587	Limonar	*Andropogon sp.*									
1589	Santander	*Andropogon gayanus*									
1590	Santander	*Brachiaria sp.*	28	57	27	3	9	4	4	10	
1591	Pto. Gaitan	*Brachiaria sp.*									
1592	Pto. Gaitan	*Brachiaria sp.*									
1594	Santander	*Brachiaria sp.*									
1595	Limonar	*Andropogon sp.*	35	63	18	4	12	3	6	9	
1596	Carimagua	*Brachiaria sp.*							13	13	
1597	Santander	*Andropogon gayanus*	71	69	21	3	5	2	8	5	
1598	Santander	*Andropogon sp.*	20	60	24	2	10	3	9	19	+
1599	Limonar	*Brachiaria sp.*									
1600	Carimagua	*Brachiaria sp.*									
1602	Santander	*Brachiaria sp.*							7	15	
1603	Carimagua	*Brachiaria sp.*							13	13	
1604	Santander	*Brachiaria sp.*	80	74	16	3	6	1	8	31	
	Brazil										
1605	Goias	*Hyparrhenia rufa*	18	59	31	2	5	3			
1606	Goias	*Mellinis minutiflora*									+
1607	Sao Paulo	*Digitaria decumbens*	78	64	22	6	6	2	0.2	5	
	Canada										
1635	Nova Scotia	Herbage	2*	56	38	2	+	4			

Yields in the sporidesmolide columns are given in g/50 g of rye except for those marked with an asterisk where the yields are mgL^{-1} of agar medium.
A + in the sporidesmolide IV column indicates that this component was detected but not integrated.

** Chemical and chromatographic analysis of epipolythiodiketopiperazines (19,5).

Discussion

The results appear to suggest that isolates of *Pithomyces chartarum* from widely distributed pastoral sites in the Americas do not produce sporidesmins in laboratory culture despite the occurrence of photosensitivity in ruminants grazing the pastures. In Australia, New Zealand and South Africa the presence of isolates of this fungus that do not produce sporidesmins (Fig 1) is well-documented (Dingley *et al.*, 1962) and indeed, in South Africa, it was thought for many years that *P. chartarum* was not part of the etiology of geeldikkop or dikoor (Kellerman *et al.*,). However, the number of isolates examined in this work from Brazil certainly exceeds the numbers that had to be investigated in South Africa and New Zealand before an organism capable of producing sporidesmin was found, but the number of isolates from a particular location was small. Another difficulty in demonstrating the production of sporidesmins by wild isolates of this fungus lies in their rapid loss of this ability after a few subcultivations in the laboratory (Dingley *et al.*, 1962). Such a loss would have to be very rapid in the case of the Nova Scotia isolates, where the cultures obtained directly from the spore-trap were subcultivated only once to provide the giant colonies for the bioassay.

We have considered the possibility that tryptophan metabolism in American isolates of *P. chartarum* might be directed towards the production of sporidesmin-D and sporidesmin-F (Jamieson *et al.*, 1969). Apart from a low toxicity (Taylor, 1971) these metabolites do not catalyse the decomposition of azide by iodine as efficiently as the di- and higher poly-sulphides, and they react very slowly with silver nitrate. Some of the isolates, particularly those from Colombia gave ultraviolet-absorbing spots on t.l.c. with the same R_F as sporidesmin-D but they could be distinguished from the latter metabolite because they had an intense pale blue fluorescence when irradiated with long wave length ultraviolet light.

One may conclude either that the density of organisms in the *Pithomyces chartarum* population in the Americas capable of producing sporidesmins is lower than it is in South Africa, New Zealand and Australia, or that the agent(s) inducing photosensitivity are not associated with this fungus, or are not related to sporidesmin.

References

Aycardi, E.R., Garcia, O., Henaw, F.J. and **Torres, B.** 1982. Fotosensibilidad experimental en bovinos en un areas tropical utilizando hongo *Pithomyces chartarum. Revista acovez,* 6: 23-24.

Bertaud, W.S., Morice, I.M., Russell, D.W. and **Taylor, A.** 1963. The spore surface in *Pithomyces chartarum. J. gen. Microbiol.* 32: 385-395.

Bishop, E., Griffiths, H., Russell, D.W., Ward, V. and **Gartside, R.N.** 1965. Spore surface depsipiptides in some *Pithomyces* species. *J. gen. Microbiol.* 38: 289-297.

Brewer, D. and **Taylor, A.** 1967. The biological properties of 3,6-epidithia-diketopiperazines: degradation of gliotoxin-B by *Bacillus subtilis* (HLX 373). *Can. J. Microbiol.* 13: 1577-1589.

Brewer, D., Taylor, A. and **Hoehn, M.M.** 1974. The antibiotic activity of cultures from fungal spores collected by a spore-trap on permanent pasture. *Can. J. Microbiol.* 20: 721-729.

Dingley, J. 1962. *Pithomyces chartarum,* its occurrence, morphology and taxonomy. *N.Z. J. Agric. Res.* 5: 49-61.

Dingley, J.M., Done, J., Taylor, A. and **Russell, D.W.** 1962. The production of sporidesmins and sporidesmolides by wild isolates of *Pithomyces chartarum* in surface and in submerged culture. *J. gen. Microbiol.* 29: 127-135.

Döbereiner, J., Tokarnia, C.H., Monteiro, M. de C da C, da Cruz L.C.M., de Carvalho, E.G. and **Primo, A.T.** 1976. Intoxicacao de bovinos e ovinos em pastos de *Brachiaria decumbens* contaminados por *Pithomyces chartarum*. *Pesq. agropec. bras. Ser. Vet.* 11: 87-94.

Done, J., Mortimer, P.H. and **Taylor, A.** 1960. Some observations on field cases of facial eczema: liver pathology and determinations of serum bilirubvin, cholesterol, transaminase and alkaline phosphatase. *Res. vet. Sci.* 1: 76-83.

Done, J., Mortimer, P.H., Taylor, A. and **Russell, D.W.** 1961. The production of sporidesmin and sporidesmolides by *Pithomyces chartarum*. *J. gen. Microbiol.* 26: 207-222.

Flynn, D.M. 1962. Facial eczema. Part I. History of the disease in Victoria. *J. Dept. Agric. Victoria (Australia)* 1962: 5-6.

Halder, C.A., Taber, R.A. and **Camp, B.J.** 1981. Absence of sporidesmin production by twelve Texas isolates of *Pithomyces* spp. *Appl. Environ. Microbiol.* 41: 212-215.

Jamieson, W.D., Rahman, R. and **Taylor, A.** 1969. Sporidesmins. Part VIII. Isolation and structure of sporidesmin-D and sporidesmin-F. *J. Chem. Soc. (C)* 1969: 1564-1567.

Kellerman, T.S., van der Westhuizen, G.C.A., Coetzer, J.A.W., Roux, C., Marasas, W.F.O., Minne, J.A., Bath, G.F. and **Basson, P.A.** 1980. Photosensitivity in South Africa. II. The experimental production of the ovine hepatogenous photosensitivity disease geeldikkop (*Tribulosis ovis*) by the simultaneous ingestion of *Tribulus terrestris* plants and cultures of *Pithomyces chartarum* containing the mycotoxin sporidesmin. *Onderstepoort J. vet. Res.* 47: 231-261.

Leigh, C. and **Taylor, A.** 1976. The chemistry of the epipolythiopiperazine-3,6-diones. *In* "Mycotoxins and other fungal related food problems". Advances in Chemistry No. 149, American Chemical Society 1976; pp. 228-275.

Liss, S.N., Brewer, D., Taylor, A. and **Jones, G.A.** 1985. Antibiotic activity of an isocyanide metabolite of *Trichoderma hamatum* against rumen bacteria. *Can. J. Microbiol.* 31: 767-772.

Marasas, W.F.O., Adelaar, T.F., Kellerman, T.S., Minne, J.A., van Rensburg, I.B.J. and **Burroughs, G.W.** 1972. First report of facial eczema in sheep in South Africa. *Onderstepoort J. vet. Res.* 39: 107-112.

Rahman, R., Safe, S. and **Taylor, A.** 1970. Separation of polythiadioxopiperazine antibiotics by thin layer chromatography. *J. Chromatog.* 53: 592-594.

Regalado, A.M. 1981. Intoxicacion por *Pithomyces chartarum* en bovinos en el partido de daireaux, Buenos Aires. *Gaceta Veterinaria* 43: 471-473.

Ross, D.J. 1960. A study of the physiology of *Pithomyces chartarum* (Berk. & Curt.) M.B. Ellis. II. Some factors influencing sporulation in culture. *N.Z. J. Sci* 3: 441-452.

Russell, D.W. 1962. Depsipeptides of *Pithomyces chartarum:* the structure of sporidesmolide I. *J. Chem. Soc.* 1962: 767-772.

Russell, D.W., Synge, R.L.M., Taylor, A., and **White, E.P.** 1962. Similarity of depsipeptides from *Pithomyces chartarum* and from pasture samples from "facial eczema" areas. *J. Chem. Soc.* 1962: 554-555.

Taber, R.A., Pettit, R.E., Taber, W.A. and **Dollahite, J.W.** 1968. Isolation of *Pithomyces chartarum* in Texas. *Mycologia* 60: 727-730.

Taylor, A. 1970. The occurrence, chemistry and toxicology of the microbial peptidelactones. Advances in Applied Microbiology, Vol. 12, Academic Press, New York. pp. 189-276.

Taylor, A. 1971. The toxicity of sporidesmins and other epipolythiadioxopiperazines. *In* Microbial Toxins Vol. 7, Academic Press, New York. pp. 337-376.

Ueno, K., Giam, C.S. and **Taber, W.A.** 1974. Absence of sporidesmin in a Texas isolate of *Pithomyces chartarum*. *Mycologia* 66: 36-362.

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SHOOT MULTIPLICATION, GROWTH AND ADVENTITIOUS ROOTING IN THREE CULTIVARS OF VITIS, IN VITRO

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An in vitro propagation scheme was established for three commercially attractive cultivars of *Vitis* spp.: "Marechal Foch", "Precose del Colmar" and "Siegfried". Terminal and axillary buds from growing vines were used to establish stock multiple shoot cultures on Murashige and Skoog medium (MS), supplemented with N^6-benzylamino-purine (BAP). In shoot multiplication trials, the highest shoot numbers were observed after 12 weeks using 5.0 μM BAP; shoot morphology was somewhat distorted at 5.0-10.0 μM BAP, but not at lower concentrations (2.5-3.75 μM BAP). In rooting trials, rapid rooting at high frequencies was observed on MS medium. Indole-3-butyric acid increased the number of roots in some trials, and rooted microcuttings could be grown on in soil.

Une méthode de propagation *in vitro* a été établie pour trois cultivars du genre *Vitis*: Maréchal Foch, Precose del Colmar, et Siegfried. On s'est servi de bourgeons terminaux et axillaires provenant de vignes en croissance pour établir des cultures de souches avec plusieurs scions sur un milieu Murashige et Skoog (MS), augmenté de 6-benzylaminopurine (BAP). Quant aux essais sur la multiplication des scions, les scions les plus nombreux ont été observés après 12 semaines dans un milieu de BAP à 5.0 μM; la morphologie des scions a été déformée aux concentrations de BAP entre 5.0 et 10.0 μM, mais non pas aux concentrations inférieures (2.5-3.75 μM). L'enracinement s'est démontré rapide et à haute fréquence dans le milieu MS. L'acide indole-3-butyrique a augmenté le nombre de racines dans certains essais, et les petites boutures enracinéssont poussé dans le sol.

Introduction

In vitro techniques for propagation of select grape cultivars are important in relation to plant germplasm preservation and rapid multiplication of both new plant introductions and of virus-indexed plants (Krul and Mowbray 1984). Although certain grape cultivars and other woody angiosperms are now commercially produced in tissue culture (Zimmerman 1985), optimum *in vitro* protocols are required to achieve efficient propagation (Reisch 1986).

Recently we reported the micropropagation of hardy "Michurinetz" grapes (Hicks, Dorey, Chiarot and Murray 1986). The present paper extends this work to three other cultivars of potential value to the Canadian wine industry which have performed well in field trials in Nova Scotia (Bishop 1984). Our objective was to devise micropropagation methods by manipulating the composition of culture media during the shoot multiplication and rooting stages.

Materials and Methods

Actively growing specimens of "Siegfried", "Marechal Foch" and "Precose del Colmar" were supplied by Sheffield Nurseries, Centreville, Nova Scotia. To establish sterile stock cultures, terminal and axilllary buds were stripped of expanding leaves and surface sterilised for 20 minutes in dilute sodium hypochlorite (3%) containing 0.1% Tween 20. Then they were rinsed three times in sterile, double distilled water and inoculated on to 20 ml semi-solid Murashige and Skoog (MS) medium (Carolina Biologicals), supplemented with inositol (1 g L^{-1}), sucrose (30 g L^{-1}), thiamine (0.1 g L^{-1}), agar (8 g L^{-1}), and 6-benzylaminopurine (BAP; 10 μM— at pH 5.8. Tubes were incubated under cool-white fluorescent lamps (75 μEinsteins $m^{-2} \cdot sec^{-1}$) at 24°C.

Explant axillary bud meristems grew, producing multiple shoot cultures. These were subcultured at 3-4 week intervals for over 20 months, in 100 mL jars on the medium described, but with 5 μM BAP.

For shoot multiplication and growth trials, microcuttings, each consisting of a shoot (1-2 cm long) bearing two expanded leaves, were harvested from the multiple shoot cultures. For each cultivar, BAP was tested at 0, 2.5 and 5 μM in the first trial (trial A), and at 0, 2.5, 3.75 and 5.0 μM in a second trial (trial B). Since 10 μM BAP produced extremely fasciated shoots in Trial A, this treatment was omitted in the second trial. For "Marechal Foch", there were three passages each of four weeks duration, while there were four passages of three weeks each, for the other cultivars. Explants of "Precose" deteriorated with passage times of more than three weeks. At each transfer, the whole explant was subcultured. After twelve weeks the total number of shoots greater than 0.5 cm in length, the fresh weight, and the dry weight of each explant were determined.

Table 1 Effect of BAP concentration on shoot number and fresh and dry weights of grape microcuttings,[1] at 12 weeks.

Cultivar	Trial	BAP (μM)	Mean Shoot No. ± C.I.[2]	Mean Fresh Wt.[3] (g) ± C.I.	Mean Dry Wt.[4] (g) ± C.I.
"Precose del Colmar"	A	0	1.5 ± 0.9	0.29 ± 0.14	0.15 ± 0.0
		2.5	22.3 ± 8.4	5.67 ± 2.55	0.62 ± 0.2
		5.0	59.3 ± 11.3	17.70 ± 5.10	1.31 ± 0.3
		10.0	39.4 ± 10.6	17.62 ± 5.20	1.18 ± 0.3
	B	0	3.3 ± 1.9	0.84 ± 0.52	0.09 ± 0.0
		2.5	19.5 ± 9.7	5.35 ± 2.55	0.49 ± 0.2
		3.75	39.0 ± 17.2	9.92 ± 5.70	0.74 ± 0.3
		5.0	55.0 ± 14.8	18.60 ± 5.30	1.19 ± 0.3
"Foch"	A	0	1.2 ± 0.4	0.23 ± 0.06	0.03 ± 0.0
		2.5	8.2 ± 3.8	2.06 ± 1.84	0.31 ± 0.2
		5.0	77.1 ± 16.,3	20.49 ± 5.10	1.26 ± 0.3
		10.0	30.8 ± 11.9	21.94 ± 5.45	1.09 ± 0.3
	B	0	1.4 ± 0.5	0.33 ± 0.12	0.14 ± 0.0
		2.5	12.8 ± 5.2	2.94 ± 1.35	0.39 ± 0.1
		3.75	38.6 ± 12.3	7.47 ± 3.40	0.69 ± 0.2
		5	68.3 ± 10.2	15.4 ± 3.7	1.02 ± 0.2
"Siegfried"	A	0	1.2 ± 0.6	0.31 ± 0.15	0.03 ± 0.0
		2.5	69.0 ± 8.4	26.63 ± 4.60	1.66 ± 0.1
		5.0	98.1 ± 19.4	40.91 ± 4.80	1.72 ± 0.2
		10.0	20.9 ± 5.1	40.91 ± 3.10	1.72 ± 0.1
	B	0	1.5 ± 0.4	0.35 ± 0.10	0.05 ± 0.0
		2.5	28.7 ± 1.0	14.50 ± 5.40	0.96 ± 0.3
		3.75	71.0 ± 16.7	28.61 ± 5.30	1.37 ± 0.2
		5.0	51.8 ± 15.4	31.12 ± 5.20	1.49 ± 0.2

[1](9-14 replicates, per BAP treatment; except for controls (6-11)).

[2]95% Confidence Interval.

[3]Initial wet weight per explant (mg): Foch: (56.1); Siegfried (36.2); Precose (74.3)

[4]Initial dry weight per explant (mg): Fosh: (5.4); Siegfried (3.6); Precose (9.7)

For rooting trials, MS salts were reduced to half-strength (Novak and Juvova 1982) and BAP was replaced with 4-(indolyl-3) — butyric acid (IBA) at 0, 0.25, 2.5, and 5.0 μM, while other constituents remained as previously described. Media were dispensed in 50 ml aliquots in 225 ml glass jars. Rooting hormone (IBA) was added to the autoclaved media through a Millipore filter (0.22 μM pore size). Microcuttings for rooting

Table II Effects of IBA concentration on root numbers.[1]

Cultivar	Trial	IBA Concentration (μM)	mean number of roots per shoot ($\pm$ C.I.)[2]
"Precose del Colmar"	A	0	2.8 $\pm$ 0.8
		0.25	3.3 $\pm$ 0.7
		2.5	8.9 $\pm$ 3.1
		5.0	11.7 $\pm$ 3.0
	B	0	3.1 $\pm$ 0.7
		0.25	5.5 $\pm$ 1.2
		2.5	10.5 $\pm$ 3.1
		5.0	12.8 $\pm$ 2.3
"Marechal Foch"	A	0	3.9 $\pm$ 1.0
		0.25	5.8 $\pm$ 1.0
		2.5	3.4 $\pm$ 1.6
		5.0	3.2 $\pm$ 1.9
	B	0	5.8 $\pm$ 1.1
		0.25	9.3 $\pm$ 2.8
		2.5	15.9 $\pm$ 2.9
		5.0	18.0 $\pm$ 3.4
"Siegfried"	A	0	5.4 $\pm$ 0.9
		0.25	5.9 $\pm$ 1.1
		2.5	9.9 $\pm$ 2.7
		5.0	3.9 $\pm$ 1.4
	B	0	3.8 $\pm$ 0.9
		0.25	4.4 $\pm$ 0.9
		2.5	7.2 $\pm$ 2.1
		5.0	12.3 $\pm$ 2.6

[1]Microcuttings grown for 12-13 days on MS medium; (35-40) replicates per treatment per experiment (except "Marechal Foch", trial A, IBA 2.5 μM N = 25).

[2]95% Confidence Interval.

Table III Survivorship of rooted grape microcuttings *ex vitro* (%).[1]

Cultivar	Previous IBA dose (ppm)			
	0	0.05	0.5	1.0
"Foch"	94	82	62	74
"Siegfried"	68	65	68	74
"Precose del Colmar"	61	65	82	67

[1]Rooted microcuttings were transferred to soil after 12-13 days *in vitro* on the rooting media. Survivorship was recorded as the percentage of healthy plants days after lid removal from germination trays at 24°C and 18 h photoperiod. N = 32-36.

came from the stock multiple shoot cultures and consisted of a stem (ca. 2 cm long), bearing the terminal bud and three expanded leaves. For each IBA concentration, there were 36 to 40 replicate microcuttings in each of two trials, lasting 12-13 days. A microcutting was recorded as having rooted if it bore at least one emergent adventitious root. Statistical analysis of the rooting data was performed using the programme GLIM (Baker and Nelder 1978). The ability of rooted microcuttings to establish *ex vitro* was tested by transferring rooted plantlets from the IBA experiments to potting soil in plastic pots seated in covered germination trays. Trays were kept at 24°C with an 18 h photoperiod, using mixed incandescent and fluorescent lamps. After two weeks, the tray lids were removed and 5 days later, the percentage of healthy surviving plants was recorded.

Results

BAP stimulated development of the axillary buds to produce multiple shoot clusters accompanied, in most instances, by roots (Fig 1). On 0, 2.5, and 3.75 μM BAP, shoot morphology was essentially normal, but at 10 μM BAP, stems were fasciated and short. They bore distorted leaves and a mass of compact green tissue at the base of the shoots. For all cultivars, 5 μM BAP produced the highest shoort numbers in trial "A" (Table I), while 10 μM depressed shoot numbers. This pattern was not reflected in the fresh and dry weight values, where there was little difference between the 5.0 and 10.0 μM treatments. In the "B" trials, 5.0 μM BAP yielded the highest numbers of shoots, except in the case of Foch and Siegfried. Fresh and dry weights were also highest using 5.0 μM BAP, with exception of Foch and Siegfried.

After an initial lag of 4-5 days, rooting percentages increased sharply, reaching 85% or greater, even without IBA treatment (Fig 2,3). There was no significant effect of either IBA concentration or cultivar on rooting frequency, no significant interaction between IBA concentration and cultivar, nor any significant difference between trials. There was, however, a significant effect of IBA concentration on the mean

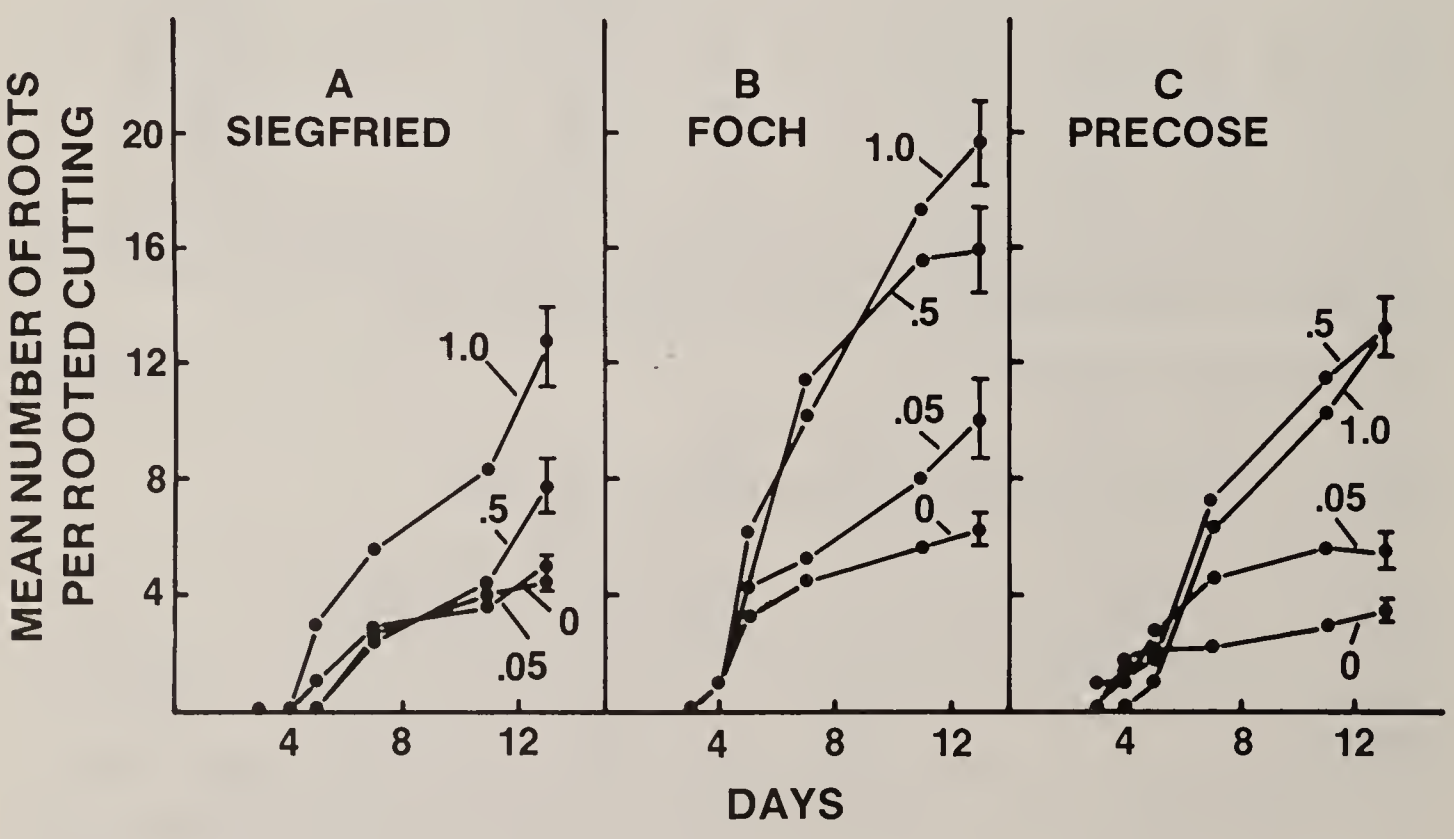

Fig 3 Rooting frequencies plotted against time.

Fig 1 Multiple shoot culture of 'Precose' after 12 weeks on MS medium with 3.75 μM BAP. Bar = 2 cm.

number of roots per shoot at 2.5 and 5.0 μM IBA (Table II), with the exception of Trial A for Siegfried and Trial B for Marechal Foch.

After transfer to soil, the rate of survival was in the range of 60-95%, but this did not depend on prior auxin treatment (Table III).

Discussion

Shoot formation using BAP has been reported for other cultivars of grape (Chee and Pool 1982; Harris and Stevenson 1982; Reisch 1986). The formation of roots on the multiple shoot cultures may be unusual in that other studies do not cite the phenomenon. Furthermore, it has been pointed out that cytokinins generally inhibit rooting in multiple shoot cultures, especially at high doses (George & Sherrington, 1984). Possibly, in our cultures, roots contributed to the development of multiple shoots, through root-derived cytokinins (Pool and Powell, 1975). The nature of the tissue mass in multiple shoot cultures has not been explored in detail, but it appears to be a compact callus. The inferior shoot quality noted above, with high levels of cytokinin, has been reported elsewhere for grapes (Goussard, 1981, 1982), but may not jeopardise propagation schemes seriously, since recovery on prolonged culture has been noted (Harris and Stevenson, 1982).

As in our study, microcuttings of 'Cabernet Sauvignon' and 'Cabernet Franc' grapes rooted readily on an auxin-free medium (Skene and Barlass, 1980). However, other cultivars in the latter study required naphthalene acetic acid (NAA) for rooting. Similar genotype-related responses to NAA have been noted (Chee and Pool, 1984).

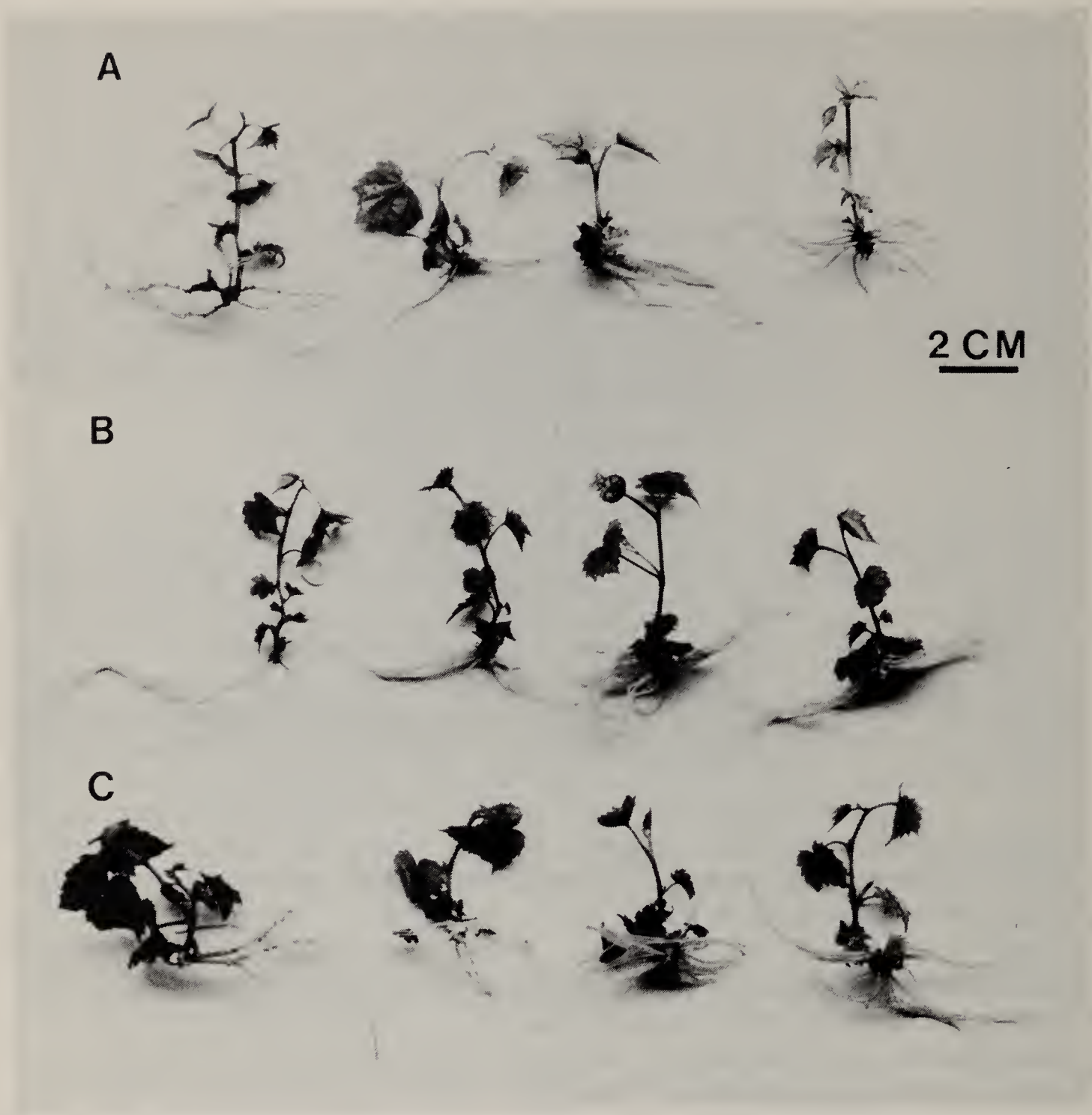

Fig 2 Rooted microcuttings of A - "Siegfried"
B - "Precose del Colmar"
C - "Marachal Foch"

In terms of the number of roots per shoot, we observed a promotive effect of high auxin concentrations, but the increase may have little significance for the develoment of micropropagation schemes. Our root numbers were generally higher than those reported (Li and Eaton 1984; Chee and Pool 1983). Overall, the present study has established the basis for a micropropagation scheme for these cultivars.

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References

Baker, R.J. and **Nelder, J.A.** 1978. The GLIM system release 3; generalised linear interactive modelling. Numerical Alogorithms Group, Oxford.

Bishop, G.W. 1984. Grape cultivar evaluation and breeding. Agriculture Canada Research Station, Kentville, N.S. Annual Report, pp. 18-20.

Chee, R. and **Pool, R.M.** 1983. The effects of growth substances and photoperiod on the development of shoot apices of Vitis cultured in vitro *Scient. Hortic. 16:* 17-27.

Chee, R. and **Pool, R.M.** 1983. *In vitro* vegetative propagation of *Vitis:* Application of previously defined culture conditions to a selection of genotypes. *Vitis 22:* 363-374.

George, E.F. and **Sherrington, P.D.** 1984. *In* Plant propagation by tissue culture. *Edited by* E.F. George and P.D. Sherrington. Eastern Press, Reading, Berks. 709 pages.

Goussard, P.G. 1981. Effects of cytokinins on elongation, proliferation and total mass of shoots derived from shoot apices of grapevine cultured *in vitro. Vitis 20:* 228-234.

Goussard, P.G. 1982. Morphological responses of shoot apices of grapevine cultured *in vitro.* Effects of cytokinins in routine subculturing. *Vitis 21:* 293-298.

Harris, R.E. and **Stevenson, J.H.** 1982. *In vitro* propagation of *Vitis. Vitis 21:* 22-32.

Hicks, G.S., Dorey, M., Chiarot, M. and **Murray, J.** 1986. Propagation *in vitro* of 'Michurinetz' grapes. *Proc. N.S. Inst. Sci. 36:* 56-61.

Krul, W.R., and **Mowbray, G.H.** 1984. Grapes. *In* Handbook of Plant Cell Culture. Vol. 2. Crop Species. *Edited by* Sharpe, W.R., Evans, D.A., Ammirato, P.V., and Y. Yamada. MacMillan. New York. pp. 396-434.

Li, J.R., and **Eaton, G.W.** 1984. Growth and rooting of grape shoot apices *in vitro. Hortsci. 19:* 64-66.

Novak, F.J. and **Juvova, Z.** 1982. Clonal propagation of grapevine through *in vitro* axillary bud culture. *Scient. Hortic. 18:* 231-240.

Pool, R.M., and **Powell, L.E.** 1975. The influence of cytokinin on *in vitro* shoot development of 'Concord' grape. *J. Am., Soc. Hortic. Sci. 100:* 200-202.

Reisch, B.I. 1986. Influence of genotype and cytokinins on in vitro shoot proliferation of grapes. *J. Am. Soc. Hortic. Sci. 111:* 138-141.

Skene, K.G.M., and **Barlass, M.** 1980. Micropropagation of grapevine. *Int. Plant Propag. Assoc. Bull. 30:* 564-570.

Zimmerman, R.H. 1985. Application of tissue culture propagation to woody plants. *In* Tissue culture in forestry and agriculture. Edited by R.R. Henke, K.W. Hughes, M.J. Constantin and A. Hollaender. Plenum Press, N.Y. pp. 165-177.

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ERRATA

In Volume 37 there are two errors in the paper by D. W. Russell and H. A. Gillis entitled "Disiloxanes and Other Artifacts from a Reversed-Phase High Pressure Liquid Chromatography Column Identified by Mass Spectrometry."

On page 14, line 3 should read:

methanol + water, 82 + *18* by volume, flowing at.....

and line 11 from the bottom of the page should read:

... possessed the general formula $C_nH_{2n+5}Si_2O$ (Table I)

PROC. N.S. INST. SCI (1989)
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INVESTIGATIONS OF THE MARINE ALGAE OF NOVA SCOTIA XVII: VERTICAL AND GEOGRAPHIC DISTRIBUTION OF MARINE ALGAE ON ROCKY SHORES OF THE MARITIME PROVINCES[1]

I. NOVACZEK and J. MCLACHLAN
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A review is given of the physical features of three coastal environments of eastern Canada: the Gulf of St. Lawrence, the open Atlantic coast, and the Bay of Fundy. Intertidal and sublittoral regions of these environments were surveyed. Vertical limits of common macrophytic algae and their relative abundances were documented and a species list compiled for sectors of each coastal environment. Differences among the three coastal enviornments in terms of species composition and vertical distributions could be related to differences in ice scour, tidal amplitude, wave exposure, intertidal slope, water temperature and geology. Sites on the outer Atlantic coast generally showed patterns of zonation corresponding with the typical pattern for the North Atlantic Ocean, whereas both Fundy and Gulf shores deviated from this pattern. The vegetation belts dominated by fucoids, red turf algae and kelp were found at progressively lower intertidal or sublittoral levels moving from the Bay of Fundy to the Atlantic and from Atlantic to Gulf shores. The changing pattern from the Fundy to Atlantic shores was correlated mainly with changing tidal amplitudes and intertidal slopes. The difference between Atlantic and Gulf shores was primarily owing to the effects of ice scour. Certain species found sublittorally in other areas occurred intertidally in the Bay of Fundy. For some species, variations in physical factors accounted for a high proportion of the variability in vertical limits. Whether this is the result of direct effects of physical factors or a consequence of changing biotic interactions requires experimental analysis. Nutrient enrichment of southwest Atlantic shores may have produced a slight increase in species diversity of this environment. Differences in the temperature regimes of the three coastal environments were clearly reflected in species compositions. Some cold-temperate to Arctic species were absent from the warmest environment, the Gulf, while certain warm-temperate species were restricted to Gulf waters.

Une revue est presentée des traits physiques de trois environnements côtiers de l'est du Canada: le Golfe du St. Laurent, la côte de l'Atlantique, et la Baie de Fundy. On a fait un relève des lieux entre les limites des marées ainsi que de ceux audessous du littoral. Les limites verticales des algues macrophytiques ordinaires ainsi que leurs abondances comparatives, ont été examinées et une liste des espèces a été préparée ayant trait aux secteurs de chaque environnement côtier. Les différences en ce qui concerne la composition d'espèces et leurs distributions verticales s'apparentent aux différences en sillonage dù à la glace, à l'ampleur des marées, à l'exposition aux vagues, à la pente entre les limites des marées, à la température de l'eau, et à la géologie. Les sites de la côte atlantique extérieure ont demontré une distribution de zones ressemblant à ceux de l'Océan Atlantique du Nord, tandis que la Fundy et le Golfe s'écartent de ce modèle. Les zones de plantes composées pour la plupart de fucus, de la varech, et des algues rouges se trouvent à de plus grandes profondeurs près de la côte en passant de la Baie de Fundy à l'Atlantique, et de l'Atlantique au Golfe. Le changement de distribution entre la Fundy et l'Atlantique se place en corrélation avec les variations d'ampleur de la marée, et des pentes entre les mers hautes et basses. La différence entre l'Atlantique et le Golfe se refère surtout aux effets du sillonage causé par la glace. Certaines espèces sont restreintes entre les limites des marées dans la Baie de Fundy, mais elles se trouvent audessous du littoral dans les autres cas. Quant à quelques autres espèces, la variation des facteurs physiques est responsable d'une haute proportion des écarts des limites verticaux. Une analyse expérimentale devrait être faite afin de comprendre si les faits ci-dessus se rattachent directement à des facteurs physiques ou à la suite d'actions réciproques biotiques inconstantes. L'enrichissement nutritif des côtes sud-ouest de l'Atlantique peut être responsable d'un petit accroissement de la diversité d'espèces de cet environnement. Les différences des régimes de température des trois environnements côtiers ont une influence sensible sur la composition des espèces. Certaines espèces situées dans les eaux froides-tempérées et arctiques sont absentes des environnements les plus chauds, e.g., le Golfe, tandis que certaines espèces des eaux chaudes-tempérées se trouvent exclusivement dans les eaux du Golfe.

[1] NRCC No. 30956

Introduction

The Maritime Provinces (Nova Scotia, New Brunswick and Prince Edward Island) have three coastal environments: the southern Gulf of St. Lawrence (hereafter the Gulf), the Atlantic coast, and the Bay of Fundy (Owens and Bowen, 1977). The algal flora is cold-temperate with warm-temperate algae occurring in some shallow embayments (Novaczek et al., 1987). The first comprehensive survey of algae in these environments was performed by Bell and MacFarlane (1933a, 1933b). Since then, algal distributions in the Bay of Fundy (Wilson et al., 1979) and Gulf of St. Lawrence (Cardinal, 1968) have been documented. There are major differences among these environments, for instance in hydrography (especially tidal amplitude), water temperature, geology, wave energy and ice scour. Differences in the vertical and geographical distributions of intertidal and sublittoral algae are therefore to be expected among the three coastal environments, either owing to physiological limitations of the algae or through the influence of physical factors on competitive interactions among algae, herbivores and predators.

Table I Abbreviations for shore levels

CD	chart datum, the level of lowest normal tides
LLWS	lower low water of spring tides
LLWM	lower low water of mean tides
MWL	mean of all hourly observations of tidal height
HHWN	higher high water of neap tides
HHWM	higher high water of mean tides
HHWS	higher high water of spring tides

The intertidal region of rocky shores in the North Atlantic (i.e. from LLWS to HHWS, see Table I for abbreviations) can in general be divided (Fig 1) into three biotic zones (Lewis, 1964; Stephenson and Stephenson, 1972). The uppermost zone, the littoral fringe, lies between the uppermost marine organisms and the upper limit of barnacles. Except for special cases, such as in severe wave exposure, this zone spans the level of HHWS. In the North Atlantic the midlittoral zone is typically occupied by dominant stands of fucoid algae, and reaches from the upper barnacle line down to the upper limit of kelp. The lowest zone, the sublittoral fringe, runs from the upper limit of kelp down to the level of LLWS and may blend indistinguishably into the shallow sublittoral region. On British shores this general pattern is known to be

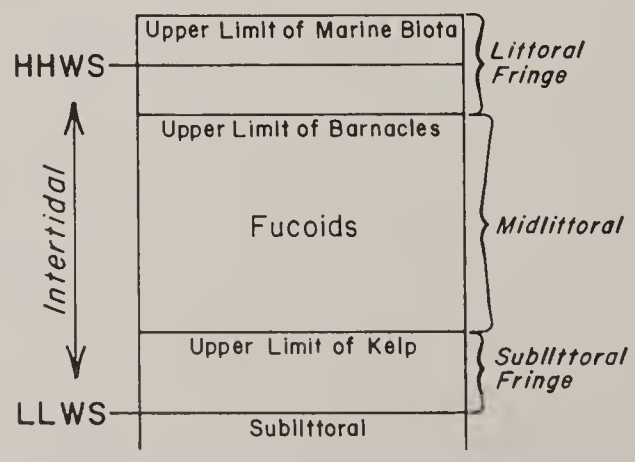

Fig 1 Schematic representation of the general pattern of zonation on rocky shores of the North Atlantic Ocean (adapted from Lewis, 1964; Stephenson and Stephenson, 1972).

sensitive to differences in wave exposure, slope, and degrees of sun and shade, any of which may alter the level of the boundaries of the zones or their species compositions.

With respect to vertical distributions of macroalgae, Stephenson and Stephenson (1954) reported that the Atlantic shores they observed conformed to the typical North Atlantic pattern, having a dominant kelp canopy defining the sublittoral fringe. In contrast, they found that the lowest intertidal level in the Gulf of St. Lawrence was either bare or dominated by *Chondrus crispus*, by fucoid algae, or by small annual brown algae. The occurrence of ice scour and the friable nature of the sandstone substratum were considered responsible for the absence of large kelp. Colinvaux (1966, 1970) found that shores of the Bay of Fundy also deviated from the expected pattern in that normally sublittoral species occurred in the intertidal region.

A few sites have recently been examined in more detail. Zonation of intertidal algae in the Gulf of St. Lawrence has been described by Lobban and Hanic (1984), and in the outer Bay of Fundy by Thomas *et al.* (1983). The sublittoral flora has been described in detail for one area in the lower Gulf (Bird *et al.*, 1983), three Atlantic coastal sites (Edelstein et al., 1969; Mann, 1972; Novaczek and McLachlan, 1986); and Passamaquoddy Bay in the outer Bay of Fundy (Logan et al., 1983). A number of surveys have been conducted to determine the extent of sublittoral laminarian cover (Wharton, 1980; Moore and Miller, 1983), the distribution of large macrophytes relative to sea urchins (Moore et al., 1986), and the extent of commercially important *Chondrus* and fucoid beds (MacFarlane, 1952, 1965; Moseley and MacFarlane, 1969; Neish and Dunn, 1971; Taylor, 1973).

In this paper we review and update the comparison of the physical features of the three coastal environments of the Maritimes and look for correlations between the changing physical features and the vertical and geographical distributions of common macroalgae in 89 study sites (Fig 2). As limits of vertical distribution can vary between adjacent transects (Southward, 1958), from year to year (Hartnoll and Hawkins, 1980), and from season to season (Druehl and Green, 1982), we concentrate on general trends in dominant species rather than describing single sites in detail.

Review of Some Features of the Coastal Environments

Hydrography and climate. The southern Gulf of St. Lawrence is a wave-dominated, microtidal environment (Owens and Bowen, 1977). The spring-tidal range is generally around 1 m but reaches almost 3 m in parts of the more wave-sheltered and current-swept Northumberland Strait. Because the tides are of a mixed semi-diurnal type, the shores regularly experience long periods of emersion (Bell and McFarlane, 1933b). Low tide sometimes coincides with mid day in summer and protective fog cover is uncommon (Table II).

Tidal amplitudes (Fig 2b) along the Atlantic coast are 1 to 2 m in Cape Breton Island (shores 11-15), about 2 m along the eastern shore of Nova Scotia (shore 10), and 5-6 m along the Fundy approaches (shores 8 and 9). The Bay of Fundy is macrotidal with the tidal range increasing to 15 m at the head of the bay. Along the Fundy approaches and outer Bay of Fundy, spring low tides are rare at mid day and often occur in near-darkness during winter months. The intertidal algae are also protected from excessive irradiance and desiccation by frequent fog (Table II). Whereas most locations in the Maritimes have maximum monthly sunshine hours in mid-summer, shore 9 experiences the most sunshine in May (Canadian Climate Normals, 1982), when the water temperature (Fig 3) is still low. Owing to swift tidal currents (1-3 m s^{-1}) bearing sediment from marshes at the head of the bay, light penetration in the Bay of Fundy is limited compared to that of the Atlantic and Gulf coasts (Table III).

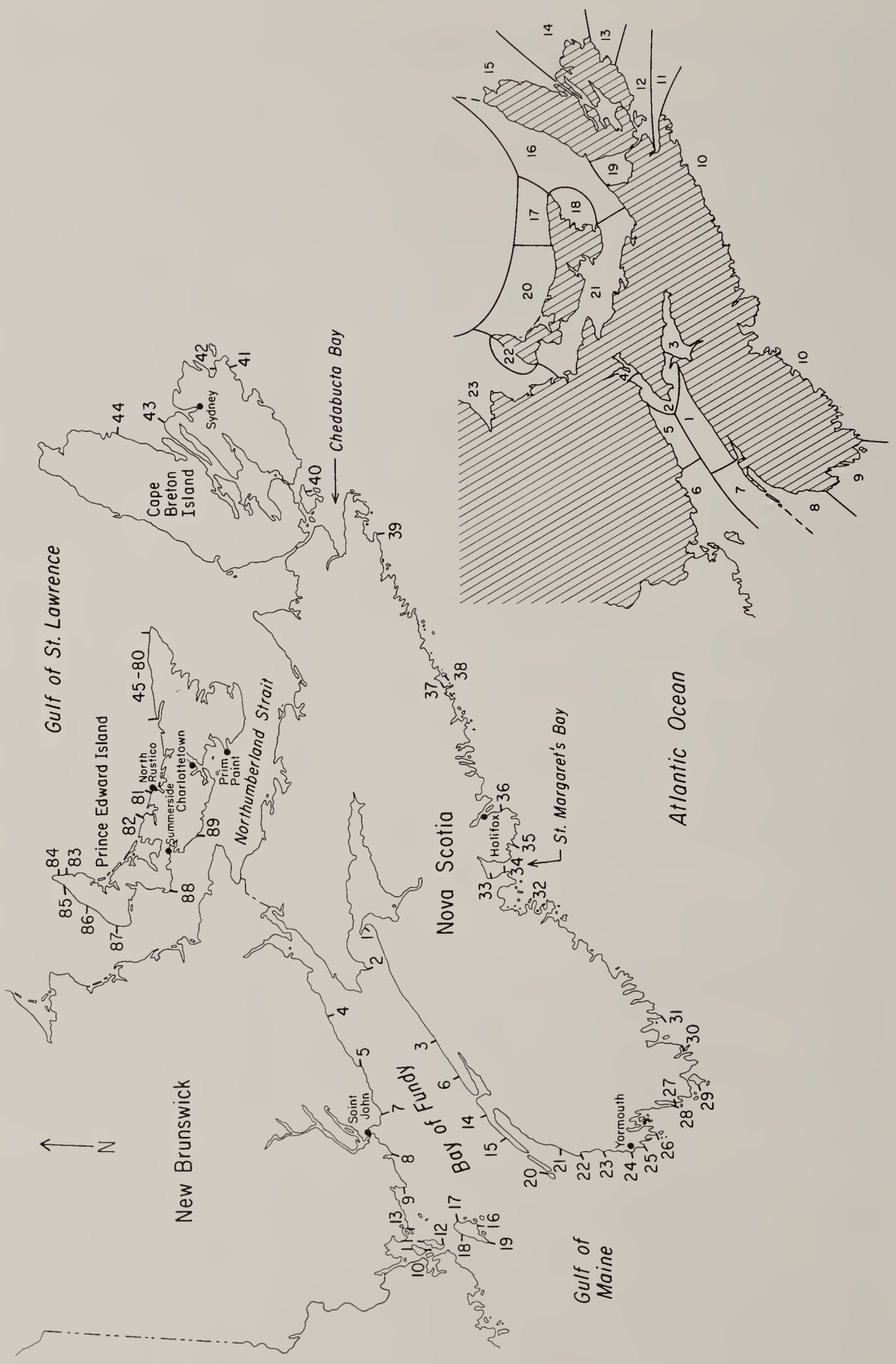

Fig 2 Maps showing a) study sites and b) the division of each coastal environment into shores (from Owens and Bowen 1977).

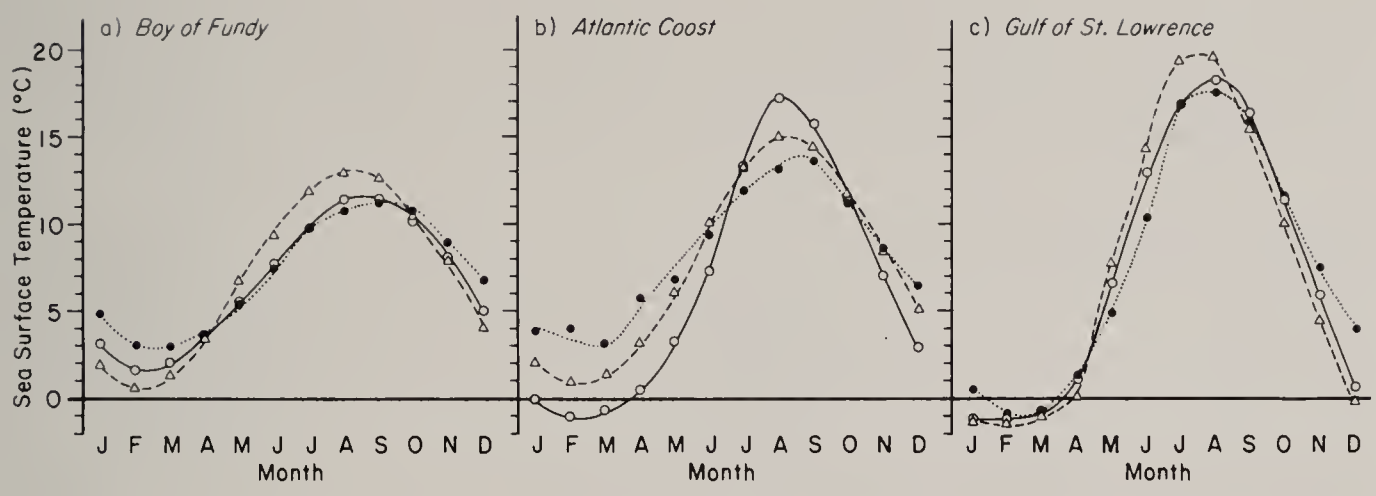

Fig 3 Representative (normal or mean) monthly sea surface temperatures in each coastal environment a) Fundy: shore 1 = ● shore 6 = ▽ , mouth of Fundy = ○ . b) Atlantic: shore 9 = ● shore 10 = ▽ , shore 14 = ○ . c) Gulf: shore 17 = ● , shore 20 = ▽ , shore 21 = ○ . (Lauzier and Hull, 1969; Dobson and Petrie, 1982; Weiler and Keeley, 1980).

Geology. The southern Gulf of St. Lawrence, except for shore 16 (northwestern Cape Breton Island), is composed of friable sandstone, commonly covered by patches of sand and gravel. On the Atlantic side of Cape Breton Island, resistant granites occur on shore 13, whereas sandstone and shale predominate on shores 12, 14, and 15. Glacial till is common over both types of bedrock (Roland, 1982).

The rocky Atlantic coast of mainland Nova Scotia consists predominantly of resistant granite and slate. In bays, on shoals and shoreward of islands, sublittoral bedrock is usually covered with glacial till.

In the Bay of Fundy, both resistant basalt and friable sandstone shores are present. Especially in the inner reaches, both the intertidal and the sublittoral regions are often covered by sand or mud.

Wind, waves and ice. In the Maritime provinces the prevailing winds are southwest and south from April to September, and north, northeast and northwest in winter

Table II Tide and fog patterns affecting desiccation in the intertidal regions of three coastal environments of eastern Canada (Canadian tide and current tables, 1984; Canadian climate normals, 1984).

	Noon low* tides yr^{-1} (Reference port)	Fog (30 yr mean) da yr^{-1} (Reference town)	Max da mo^{-1} (Reference town)
FUNDY			
shore 6	0 (St. John)	106	> 10, June-Sept. (St. John)
ATLANTIC			
shore 9	0 (Yarmouth)	118	> 10, May-Sept. (Yarmouth)
shores 10, 14	12, Sept.-June (Halifax)	80-84	> 10, May-Aug. (Halifax, Sydney)
GULF			
shores 20-21	9, Apr.-Aug. (Rustico)	47	< 10, Jan.-Dec. (Charlottetown)

* Below LLWM between 1000 h and 1300 h.

Table III Light attenuation coefficients (Kd) measured in the surface water layer (< 30 m depth) of three coastal environments (Platt and Irwin, 1970; Marine Ecology Lab, 1980; Thomas et al., 1983; Logan et al., 1983; Prouse, 1983; C. Hudon, unpubl. data).

	Kd (m^{-1})	Month
FUNDY		
shore 2	0.6-2.4	Feb., Mar., Aug.
shore 6	0.3-0.45	Aug., Oct.
shore 7	0.3-0.6	Feb., May, Aug.
	0.5-1.4	July, Aug.
ATLANTIC		
shore 10	0.1-0.25	Jan.-Dec.
	0.2-0.4	Jul., Aug.
GULF		
shore 17	0.1-0.6	June-Aug.
shore 19	0.1-0.4	April-Nov.

(Owens and Bowen, 1977). Parts of the Atlantic and Fundy coasts facing south and southwest are consequently most wave-exposed during summer. In winter the prevailing northwest winds blow offshore over most of the coastline. The maximum wave heights (>7 m) are nevertheless recorded in winter on the outer Atlantic shores (shores 10-13) because the winter winds of maximum velocity are from the east and northeast (Owens and Bowen, 1977) across the Atlantic Ocean. Waves reach 6 m high on the Fundy approaches, can be 5.8 m on shore 17 in the Gulf and rarely exceed 4 m within the Bay of Fundy (Owens and Bowen, 1977).

Sea ice moderates wave energy in winter but by its motion it can denude intertidal and upper sublittoral regions. In the southern Gulf of St. Lawrence, ice is present for three to five months (Owens, 1976). Atlantic shores 12 and 15 have onshore ice for two to four months. Except for sheltered bays, the remainder of the Atlantic and Fundy coasts is virtually ice-free (Stephenson and Stephenson, 1954), experiencing only sporadic damage from moving floes.

Another factor moderating wave energy is the presence of shoals and offshore islands. Along most of the outer Atlantic and Fundy shores, the 18m depth contour is less than 2 km offshore (Canadian Hydrographic Service Charts, Marine Science Branch, Ottawa). Exceptions are found at the head of the Bay of Fundy and round southwestern Nova Scotia (shore 9) where shoals less than 18 m deep extend outward 6-12 km. Offshore islands shelter much of the northern half of shore 10. In the southern Gulf, shoals less than 18 m deep extend at least 2 km, and often 4-6 km offshore.

Wave shock in intertidal regions is also related to slope, with less stress-tolerant species being confined to gentle slopes on wave-exposed shores (Lewis, 1964). Our survey sites in the Bay of Fundy had significantly steeper intertidal slopes (tested by analysis of variance, $p = 0.003$) than the Atlantic sites.

Water temperature, salinity and nutrients. Minimum average monthly sea surface temperatures vary from -1.8°C on Gulf and ice-bound Atlantic shores to 3°C in parts of the Bay of Fundy and Fundy approaches (Fig 3). Because of upwelling at the mouth of the bay, Fundy surface-water temperature reaches only 12-15°C in summer, compared to maxima of 17-18°C on the Atlantic coast and over 20°C (26°C in bays) in the Gulf. The Gulf is strongly stratified in summer (Steven, 1974): at 20 m depth the temperature remains 5-8°C below that at the surface (Marine Ecology Laboratory,

1980; Bird *et al.*, 1983), and incursions of cold water may reach upwards to 10 m depth. Atlantic waters are also stratified in summer, temperatures at 20 m remaining below 10°C (Drinkwater and Taylor, 1982). In contrast, Fundy waters are well mixed, with only a 1-3°C decrease in the top 20-40 m in summer (Gran and Braarud, 1935; Bailey, 1954).

Salinity varies little except in estuaries, but can be lower in the Gulf surface layer (26-32 °/oo) than in the other coast waters (30-33 °/oo) (Bailey, 1954; Loring and Nota, 1973; Taylor, 1975; Lobban and Hanic, 1984).

Whereas phosphate is always measurable and found in similar concentrations in all three coastal environments, the concentration of nitrate is greatest in winter-spring in both Gulf and eastern Atlantic waters, and drops to very low levels in summer (Platt and Irwin, 1970, 1972; Coote and Hiltz, 1975; Chapman and Craigie, 1977; Coote and Yeats, 1970; Chapman and Gagné, 1980; Gagné and Mann, 1981; Probyn and Chapman, 1983). Atlantic waters have a longer-term and more pronounced nitrogen minimum concentration than Gulf waters because the Gulf is relatively enriched by upwelling and by input from the St. Lawrence River (Garret and Loucks, 1976; El-Sabh, 1976; Sutcliffe et al., 1976). There is substantial upwelling along the Fundy approaches. Consequently, these shores and the Bay of Fundy have relatively high and stable nutrient levels, with summer nitrate concentrations being an order of magnitude higher than on other Atlantic and Gulf shores (Gran and Braarud, 1935; Lauzier, 1967).

Herbivory. Any discussion of algal distribution must take account of the importance of herbivores. The sea urchin *Strongylocentrotus droebachiensis* can denude the sublittoral and lower intertidal regions, leaving behind only crustose corallines and a few non-preferred food species such as *Agarum cribrosum, Desmarestia viridis, D. aculeata* and *Ptilota serrata* (Himmelman, 1980; Wharton, 1980; Chapman, 1981; Wharton and Mann, 1981; Johnson, 1984). Sea urchins may be excluded from shallow water by wave action, low salinity or soft sediment. During our survey period, dense concentrations of sea urchins were present inside the Bay of Fundy, along the east coast of Cape Breton Island and below 7-12 m depth in the Gulf (Appendix 1). Sea urchins were uncommon on the southwest Atlantic shore. In the past, sea urchin numbers have varied among and within the coastal environments (Stasko *et al.*, 1977; Stasko et al., 1980; McPeak, 1980; Steele, 1983). In the early 1980's there was a well-documented transition on the Atlantic eastern shore (shore 10) from urchin-dominated barrens to macroalgal beds (Moore and Miller, 1983; Miller and Colodey, 1983; Schiebling, 1986; Miller, 1985; Novaczek and McLachlan, 1986; Moore *et al.*, 1986). We surveyed shore 10 after this transition.

Smaller herbivores such as gastropods, amphipods, isopods, limpets and chitons are present (Steele, 1983; Logan *et al.*, 1983) and no doubt affect the local distributions of certain algae (Lubchenco, 1980, 1983; Johnson and Mann, 1986), but the relative abundances of such animals in the various coastal environments have not been documented.

Survey Methods

Intertidal and sublittoral surveys were conducted in spring and summer between 1977 and 1985 (Appendix 1). Some of the Gulf survey data (sites 45-80) have previously been presented in part (Bird *et al.*, 1983). These 35 transects were selected, on the basis of absence of widespread sand cover, from a set of 50 transects performed at 1 km intervals. All other study sites were at least 30 km apart (Fig 2a).

Intertidal surveys were undertaken during calm weather and spring tides. Surveys were restricted to unpolluted marine sites with hard substrata and road access. To

obtain data from shores of comparable wave exposure in all environments, most transects sere set on moderately wave-exposed shores. Each site was assigned a wave exposure index (Table IV).

Table IV Wave exposure index applied to survey sites

Index	Criteria
1 Sheltered	Not exposed to open ocean in any direction; maximum fetch < 10 km.
2 Semi-sheltered	No NW, SW or E exposure to open ocean except over shoals of < 18 m depth and > 5 km width; open to > 10 km fetch in other directions.
3 Semi-exposed	In an enclosed bay or gulf, with a fetch of > 10 km; open to the SW, E or NW over an angle of < 20° or moderated by shoals 2-5 km wide.
4 Exposed	Exposed to the SW or NW over an angle of at least 20°; 18 m contour < 2 km offshore.

For intertidal observations, a transect line marked at 1 m intervals was run on a compass bearing from the uppermost marine vegetation down to about 1 m below the level of low tide. Relative heights of the upper, and when possible the lower, limits of distribution of all common macroscopic algal species, major breaks in shore slope, and the low water mark were measured using a Geotec surveyor's level and stadia rod. Estimates were made of the total and relative abundances of macroalgae along the transect line. Species were considered dominant if they provided the greatest percentage cover, either in the canopy or the understory. Where several species were roughly equal in percentage cover, they were recorded as co-dominant. Where total foliose algal cover was less than 20%, no species was considered dominant. The points on the transect at which changes occurred in the relative dominances of species were recorded. Rock-pool inhabitants were noted separately. Small or taxonomically difficult species were collected for identification in the laboratory.

Survey data were converted to heights above chart datum, using as a reference point the predicted height of the low tide at the nearest reference or secondary port in the Canadian Tide and Current Tables and interpolating as required. The study sites rarely coincided with reference ports, and owing to variations caused by atmospheric pressure and wave action, error for intertidal levels is estimated to be about 0.3 m. Widdowson (1965) found that variation in surveyed algal limits was 0.1-0.2 m.

The upper and lower distributional limits of algae were plotted or tabulated relative to tidal levels (Table I). These tidal levels reflect the percentage of time spent exposed to the air (Lewis, 1964; Underwood, 1978) but are not meant to represent significant discontinuities in the intertidal environment.

Sublittoral observations were performed by divers swimming a compass course perpendicular to the shore, starting from the low-water level. Sublittoral transects were continuations of intertidal transects except where the intertidal region was barren or too steep to survey. In most cases the diver swam seaward to 20 m depth, noting the depth ranges of macrophytes, the relative abundances of species and the type of substratum. Depths were measured using a simple pressure gauge and were therefore inaccurate at 0 to 10 m depth, and accurate within about 1 m in deeper water. Our distributional limits for sublittoral species are therefore accurate only to 1-2 m. Separate plant collections were taken within 2-5 m depth intervals, or, in the case of Gulf sites, at randomly numbered points on a transect line. In treacherous

water or on a wide shoal, divers were dropped at intervals from a boat. Depths were converted to depth below chart datum (= CD) using tide tables and merogram (Hanic, 1974).

As many field workers were involved in this survey, variability in assessment of relative abundance was inevitable. Where broad areas were dominated by 100% cover from one or two species, as in the case of stands of intertidal fucoids or sublittoral kelps, there was no problem with the abundance scale. In sublittoral areas not occupied by dense kelp beds, use of a subjective abundance scale was particularly difficult. Distributional patterns in such areas must be viewed in general terms, with little importance given to minor differences.

Nomenclature for subdivisions (shores) of each coastal environment follows Owens and Bowen (1977) except that their southern Fundy and eastern Atlantic shores are further subdivided (Fig 2b). Our species list (Appendix 2) includes species that were rarely, if ever, dominant or were seasonal in occurrence; these will not be considered in detail. In Appendix 2 shores have been grouped in sectors on the basis of general coastal aspect and wave exposure.

Upper and lower limits of common macroalgae were analysed using analysis of variance for unbalanced designs and stepwise linear regression, from the SAS statistical package (SAS Institute Inc., 1987). As it is likely that many relationships are not linear, linear regression gives conservative indications of the correlations of vertical limits with the tested variables.

Results

Geographical distributions of algae. We identified 189 algal species; 77 red, 68 brown and 44 green. These macroalgae represent roughly half of the known algal taxa of eastern Canada (South, 1984). To avoid misrepresentation of distributions, the species recorded at various shore levels in each sector of the coastline have been tabulated (Appendix 2) together with data (in brackets) from sources other than the present survey. The macroalgal flora of the Atlantic coastal environment, with 177 species, was found to be more diverse than that of the Gulf (158 species) or the Bay of Fundy (153 species). For the eastern Atlantic coast alone (shores 10-15, exclusive of the Fundy approaches), 168 species were recorded. We found only 118 species on shores 1-5 of the inner Bay of Fundy.

The majority of species were found in all three coastal environments. The warm-temperate species *Chondria baileyana* and *Chaetomorpha aerea* were found only in the southern Gulf, as were a number of species having a more restricted southern distribution but with their northern limits in or just north of the Gulf (*Striaria attenuata, Ascocyclus distromaticus, Delamarea attenuata,* species of *Giffordia* and *Vaucheria,* and *Audouinella dasyae*). Others of southern or more widespread distribution were present on the Gulf and Atlantic shores but not in the cooler (in summer) Bay of Fundy (*Derbesia vaucheriaeformis, Stylonema alsidii,* species of *Callithamnion, Polysiphonia elongata* and *P. subtilissima*). Some species having southerly distributions (*Ceramium deslongchampii, Gloiosiphonia capillaris, Spongomorpha spinescens, Monostroma pulchrum, Porphyra miniata, Porphyra leucosticta, Polysiphonia lanosa, Phymatolithon lenormandii*) were not found in the southern Gulf but have been recorded from the middle of the Gulf, on the Magdalen Islands or Gaspé Peninsula (Cardinal, 1968). *Scinaia forcellata,* another southern species, has been recorded only in the Gulf and Fundy approaches. *Furcellaria lumbricalis* presently occurs only in the Gulf, on Atlantic shore 12 and on the northeast tip of shore 10, but its distributional limits are expanding in western Prince Edward Island (McLachlan, pers. obs.). *Fucus serratus* had a distribution similar to that of *F. lumbricalis,* but it has

also been found in abundance on the Fundy approaches (Edelstein et *al.*, 1972) and very recently at one site in Lunenburg County (McLachlan, pers. obs.). *Alaria esulenta* and *Ascophyllum nodosum* were absent from our Gulf sites, although *A. nodosum* is known from some localities in this coastal environment (Lobban and Hanic, 1984). The foliose phase of *Mastocarpus stellatus* was absent from the Gulf although the crustose phase, *Petrocelis cruenta,* has been recorded (Bird et *al.*, 1983). Foliose plants of this species were present occasionally on the Atlantic coast and often dominant in the Bay of Fundy (Fig 6).

A number of cold-temperate to Arctic species (*Ralfsia fungiformis, Elachista lubrica, Enteromorpha groenlandica, Audouinella purpurea*) were absent from the southern Gulf but present on the colder Atlantic or Fundy shores. However, a few cold-temperate to Arctic species found on Atlantic or Gulf shores (*Dilsea integra, Halopteris scoparia, Stictyosiphon griffithsianus, S. tortilis*) were not found in the Bay of Fundy. Four species (*Melanosiphon intestinalis, Scytosiphon dotyi, Blidingia chadefaudii* and *Spongomorpha sonderi*) were found only in the Bay of Fundy and/or Fundy approaches.

Vertical distributions of algae: general intertidal patterns. Despite considerable variation in both the vertical limits of species and species compositions of each shore (Appendix 3) some trends could be discerned in distributional patterns. There was an increase in species diversity moving from the head to the mouth of Fundy and from eastern to western Atlantic sites (Fig 4). The intertidal regions of all Gulf sites and Atlantic sites 42-44 (Fig 2a, Appendix 1) were greatly or completely denuded by ice scour. The sublittoral region was relatively impoverished in the Bay of Fundy and relatively diverse in the Gulf (Fig 4).

On those sites having significant intertidal vegetation, three zones were generally recognisable, conforming to the typical zonation pattern for North Atlantic shores (Fig 1). The littoral fringe was dominated, in season, by various species, including *Enteromorpha, Porphyra, Ulothrix, Prasiola, Urospora,* cyanophytes and lichens. The midlittoral zone was dominated by 80-100% cover of one or more fucoids. Kelp species, the indicators of the sublittoral fringe, were not always present intertidally.

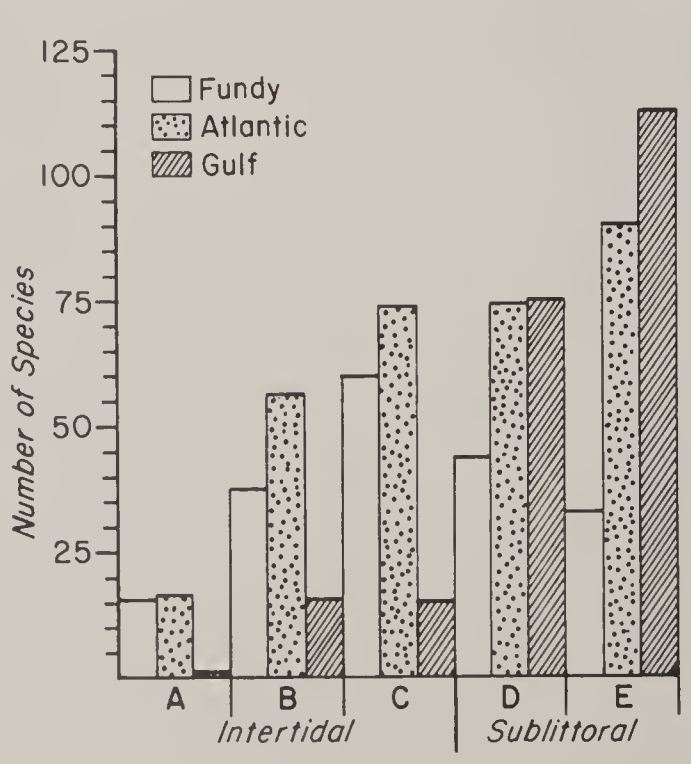

Fig 4 Numbers of species at different intertidal and sublittoral levels in three coastal environments of the Maritime Provinces. A = above HHWN; B = HHWN to LLWN; C = 0 - 2 m depth; D = 2-20 m depth.

Table V The average upper and lower limits (relative to HHWS, where HHWS = 100) of common species of *Fucus* and *Ascophyllum* found intertidally along the Bay of Fundy and Atlantic coasts.

		Fundy		Atlantic			
				Fundy approaches		Eastern Atlantic	
Species		upper	lower	upper	lower	upper	lower
F. spiralis	avg	89	84	72	58	83	76
	SE (n)	3.4 (5)	3.5 (5)	2.9 (7)	4.77 (7)	3.5 (9)	4.4 (9)
F. vesiculosus	avg	78	26	69	20	76	25
	SE (n)	2.0 (20)	2.0 (20)	2.8 (9)	4.6 (9)	3.9 (11)	4.7 (11)
A. nodosum	avg	76	23	68	12	76	25
	SE (n)	3.0 (20)	1.7 (20)	2.7 (9)	2.5 (9)	3.9 (11)	4.6 (11)
F. evanescens	avg	33	19	17	5*	28	5*
	SE (n)	3.0 (14)	1.6 (14)	5.9 (7)	1.5 (5)	4.8 (9)	6.3 (8)
F. serratus	avg	nd	nd	32	5	16	< CD
	SE (n)			22 (2)	5 (2)	(1)	

* only for sites where limit was > CD.
SE = standard error; nd = no data; < CD = below chart datum.

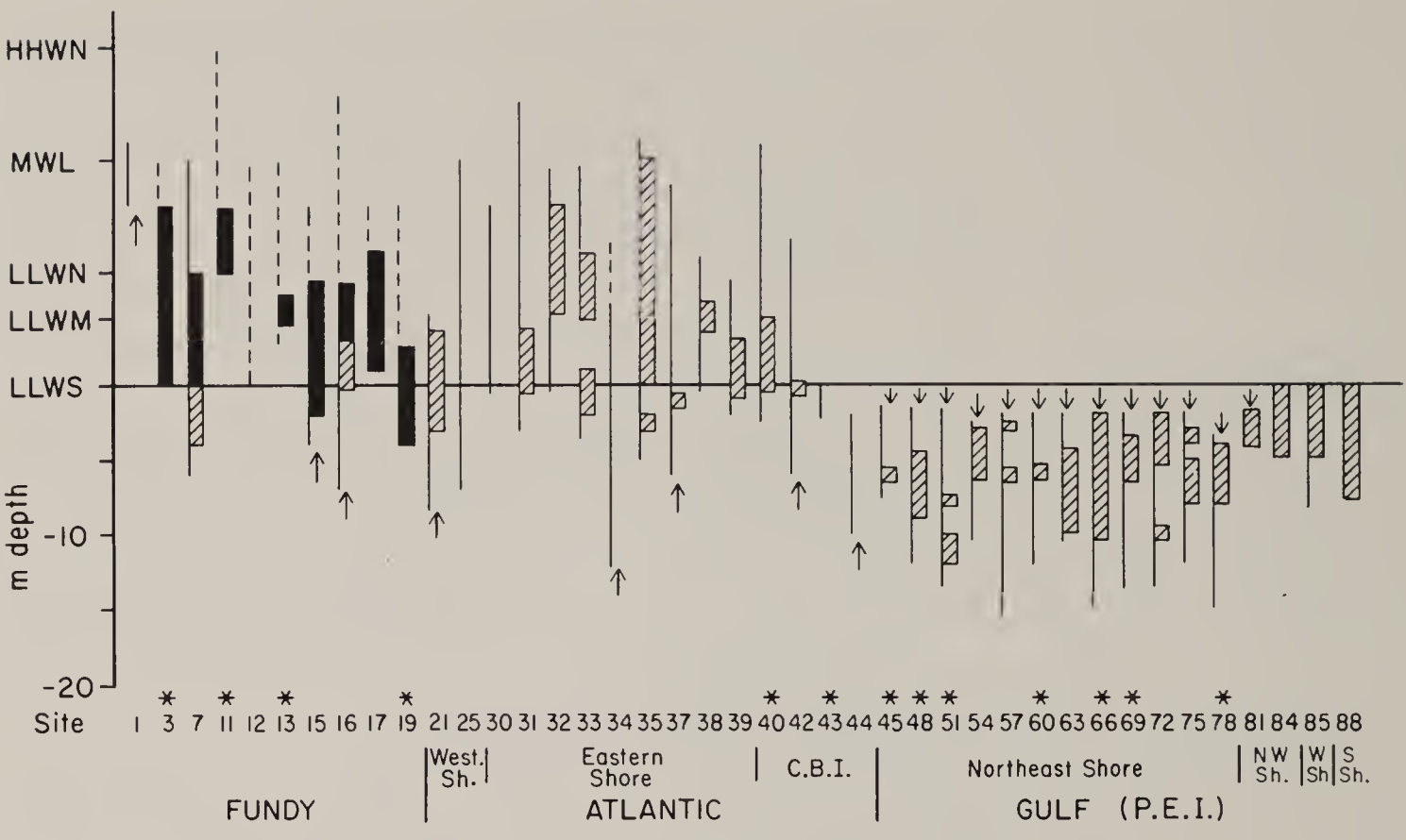

Fig 5 Limits of *Chondrus* and *Mastocarpus* at selected sites. Arrows = junctures with soft substrata; * = lower limit bordered by sea urchins. Thick bars denote dominance; thin lines, presence. *Chondrus* = diagonal bar and solid line; *Mastocarpus* + *Chondrus* = solid bar and dashed line.

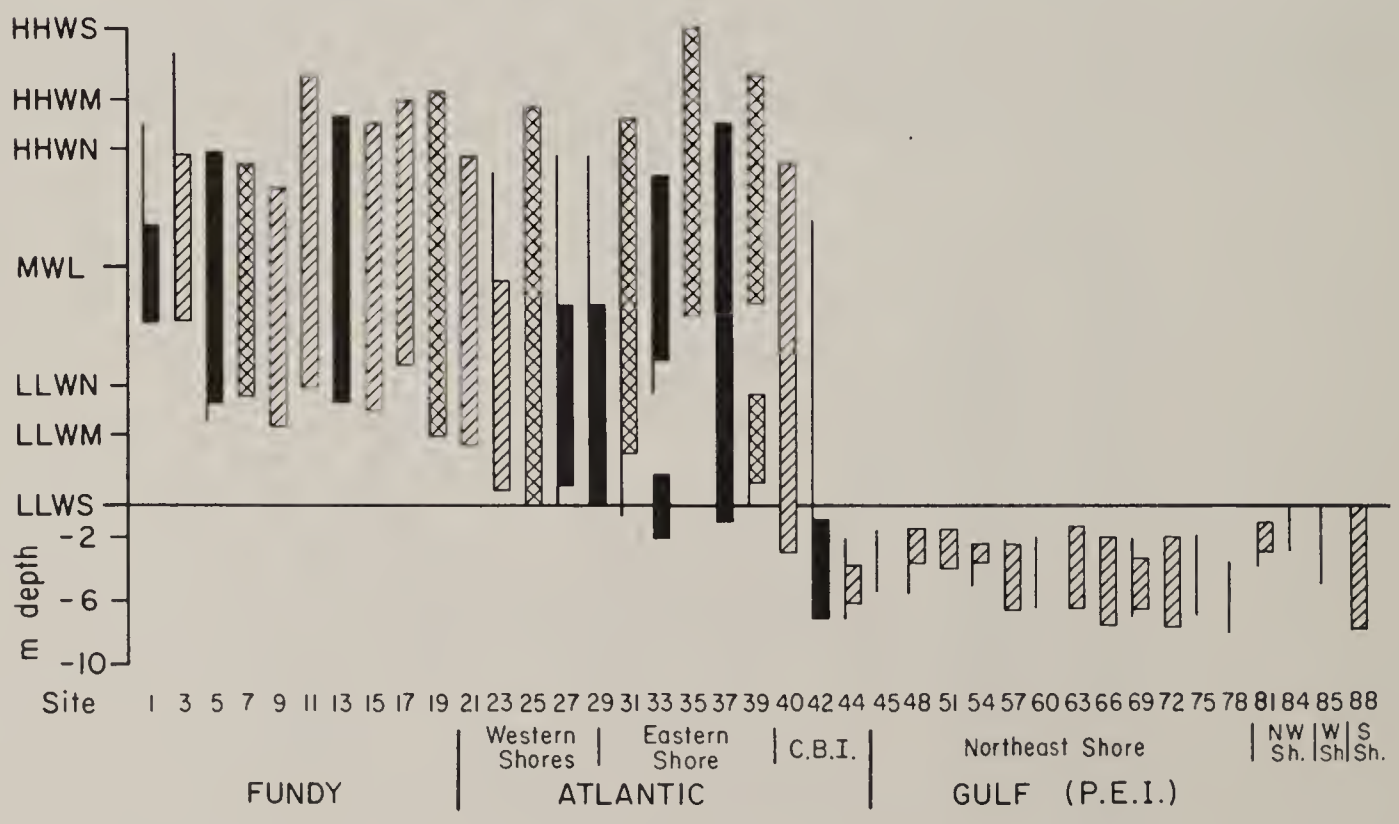

Fig 6 Limits of the fucoid belt. Thick bars denote dominance; thin lines, presence. Patterns indicate wave exposure index of site (Table IV): sheltered or semi-sheltered = solid; semi-exposed = diagonal; exposed = cross-hatched.

Depending upon conditions, a red algal turf, fucoids or ephemeral species could dominate or co-dominate the lowest intertidal region.

The fucoid species consistently occupied particular shore levels relative one to another (Table V). *Fucus spiralis,* and *F. distichus,* when present, occupied the highest level, alone or together with *F. vesiculosus* or *Ascophyllum nodosum* or both. *Fucus vesiculosus* and *A. nodosum* dominated the middle of the intertidal region and often graded, at their lower limits, into more open stands of *F. evanescens* or *F. serratus.* Because vegetative *F. evanescens* is difficult to distinguish from evesiculate *F. vesiculosus,* upper limits of this species were likely underestimated. All fucoid species, except the relatively rare *F. distichus* and *F. serratus,* occurred in all conditions of wave exposure. There was a tendency, however, for *F. vesiculosus* to be the most abundant species at the most wave-exposed sites (site 35, Appendix 3), while *A. nodosum* could be relatively abundant on more sheltered sites (sites 26, 30, Appendix 3). Fucoid holdfasts occupied little primary space. *Chondrus crispus* or *Mastocarpus stellatus* or both occupied the understory, reaching above MWL in some localities (Fig 5). Holdfasts of these understory species, the encrusting algae *Hildenbrandia rubra* and *H. Crouanii* and marine lichens (species of *Verrucaria*) occupied increasing amounts of primary space moving seaward.

Sites in the Bay of Fundy and on shore 8 of the Fundy approaches varied from most Altantic sites in that fucoids did not exclusively dominate the lower reaches of the midlittoral zone (Fig 6). Except where the low intertidal was covered by sediment (sites 1, 4) or denuded by sea urchins (sites 10, 13), the gap between that area totally dominated by fucoids and the upper limit of kelp was occupied by a dense turf of *Chondrus crispus* or *Mastocarpus stellatus* or both (sites 2-21, Appendix 3).

Upper and lower limits of distribution in the intertidal region: variation within and among coastal environments. Progressing from the Fundy to Atlantic to Gulf environments, the upper limits (in m relative to CD) of major belts of vegetation dominated by fucoids, turfing red algae (*Chondrus* and *Mastocarpus*) and kelp shifted downwards (Table VI). This was predictable because the reduction in tidal amplitude (from 15 m to 2 m) from the Bay of Fundy to the outer coast reduces the possible vertical extent of all marine organisms, while ice scouring in the Gulf limits the upward extent of macrophytes.

The relationships between various variables and both upper and lower limits of distribution of common macrophytes were tested by linear regression analysis. Regression analyses were performed firstly on all data (Table VIIa) and then on the subset of sites having intertidal algae (Table VIIb). The upper and lower limits of fucoids, and the upper limits of red turf species and of kelps were strongly related to tidal range and ice scour. On ice-scoured sites the larger macroalgae were confined to the sublittoral region, and the intertidal region was either bare or bore dense populations of small ephemeral algae and occasional juvenile fucoids (site 42, Appendix 3).

The fucoid zone contracted where there were unstable substrata in either the upper intertidal or sublittoral regions (Table VII). The lower limits of fucoids were significantly correlated with the upper limits of red turf algae. Red turf species tended to have higher upper limits on sites having sublittoral sand borders, these being predominantly Atlantic sites. Upper limits of intertidal kelp were positively correlated with wave exposure and intertidal slope. On sites that were sheltered, semi-sheltered or gently sloping, kelp could be absent from the intertidal region (sites 30, 34a, 37, 40, Appendix 3).

Although a difference in absolute height of algae on the shore is an obvious feature of changing tidal range, variation in the limits relative to tidal levels (expressed as % HHWS) is not an obvious consequence. We found that proceeding from the Bay of

Table VI Limits (m from CD) of common macroalgae in each coastal environment.*

	Fucoids		Red turf		Kelp		Agarum	Phyllophora spp.
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Upper
FUNDY								
mean	7.2	2.0	3.8	-0.9	1.3	-3.1	-0.6	4.4
SE	0.5	0.3	0.4	1.0	0.1	0.7	1.3	2.8
n	20	20	19	12	16	10	11	
ATLANTIC SHORES								
8-9								
mean	3.6	0.3	1.5	-3.3	0.4	-6.0		
SE	0.2	0.1	0.2	3.7	0.1			
n	9	7	9	2	8	1		
10-13								
mean	1.7	-1.3	1.0	-3.6	0.1	-9.3	-7.3	-4.3
SE	0.1	0.5	0.1	1.1	0.2	1.1	0.9	2.2
n	11	10	11	10	12	11	9	7
14-15								
mean	-0.3	-6.3	-0.5	-6.7	-2.0	-7.7		
SE	0.9	0.3	0.8	1.8	0.0	1.2		
n	3	3	3	3	3	3		
GULF								
mean	-1.9	-5.9	-1.8	-10.8	-5.2	-14.8	-14.7	-7.0
SE	0.2	0.2	0.2	0.5	0.3	0.3	0.8	0.5
n	41	41	45	45	45	44	7	40

* Abbreviations as in Table V.

Table VII Stepwise linear regression modelling of vertical limits (m from CD) of common macroalgae.

Species (variables tested)	Type of Limit	(n)	Significant Variable	Effect	Partial R^2	Model R^2	Prob. > F
a) All sites							
Fucoids	upper	(84)	t	+ve	0.91	0.91	0.0001
(tigwh)			i	-ve	0.05	0.95	0.001
			h	-ve	0.001	0.95	0.15
Fucoids	lower	(76)	i	-ve	0.81	0.81	0.0001
(tigwlubRK)			t	+ve	0.06	0.87	0.0001
			b	-ve	0.01	0.88	0.01
			R	+ve	0.01	0.89	0.02
Red turf	upper	(87)	t	+ve	0.74	0.74	0.0001
(tigwlub)			i	-ve	0.07	0.81	0.0001
			b	+ve	0.01	0.82	0.05
Kelp	upper	(84)	i	-ve	0.71	0.71	0.0001
(tigwlub)			t	+ve	0.07	0.72	0.11
b) Sites with intertidal algae							
Fucoids	upper	(41)	t	+ve	0.95	0.95	0.0001
(tigwhs)			h	-ve	0.004	0.96	0.05
Fucoids	lower	(29)	t	+ve	0.70	0.70	0.0001
(tigwlsR)			R	+ve	0.10	0.80	0.001
Red turf	upper	(40)	t	+ve	0.63	0.63	0.0001
(tigwlubs)			u	+ve	0.03	0.66	0.08
			g	+ve	0.02	0.68	0.11
Kelp	upper	(36)	t	+ve	0.39	0.39	0.0001
(tigwlubs)			i	-ve	0.16	0.55	0.001
			s	+ve	0.09	0.64	0.01
			w	+ve	0.07	0.71	0.01
			l	+ve	0.02	0.73	0.13

Abbreviations: Fucoids = *Fucus* and *Ascophyllum*; Red turf = *Chondrus* and *Mastocarpus;* Kelp = *Laminaria*, *Alaria*, and Saccorhiza; t = Tide range of spring tides in m; i = Ice scour; u = sea urchins; g = geology; w = wave exposure (Table II); h/l = presence of sand in upper or lower intertidal zone; b = presence of sublittoral sand border; s = slope ($m\ m^{-1}$); R = Upper limit of red turf spp; K = upper limit of kelp spp.

The variables tested in each case are indicated in brackets.

Fundy towards the Atlantic coast, the lower limit of the composite fucoid belt and the upper limit of kelps shifted downward relative to tidal levels (Figs 6-7).

Within the Bay of Fundy, on both north and south shores and regardless of wave exposure or geology, the lower limit of the fucoid belt was generally above LLWM and occasionally was above LLWN (Fig 6; sites 2-19, Appendix 3). Along the Fundy approaches (Fig 6; sites 21-26, Appendix 3) the fucoid belt progressively dropped to as low as LLWS. On the east Atlantic shore (Fig 6; sites 30-44, Appendix 3), fucoids commonly extended into the sublittoral region where they formed mixed stands with laminarians, *Chondrus* and various red algae to 1-2 m below chart datum (CD). In a statistical comparison of intertidal limits in the Bay of Fundy, Fundy approaches and eastern Atlantic shores, differences among the environments in both upper and lower limits of the fucoid belt (relative to tidal levels, see Table V) were significant (ANOVA, $p < 0.05$), as were the differences in the upper limits of *Fucus spiralis* and *F. vesiculosus* and the lower limits of *F. spiralis, Ascophyllum nodosum,* and *F. evanescens*. Differences in the upper limits of kelps were non-significant ($p = 0.06$). On both Fundy and Atlantic shores, kelp species occurred as high as LLWN (Fig 7), but only in the Bay of Fundy formed dominant canopies to this level.

Regression analyses showed that the upper limits of all fucoid species (relative to tidal levels) were significantly and positively correlated with intertidal slope, and the upper limit of the fucoid belt as a whole was also correlated with wave exposure (Table VIII); in other words, these species occurred at higher levels on steep or wave-exposed shores. The upper limit of the fucoid belt lay above HHWN under semi-exposed and exposed conditions and reached HHWS only at Prospect (site 35, Appendix 3), which was both steep and wave-exposed. The tendency for the upper limit of the fucoid belt to be higher on hard bedrock than on sandstone or shale was not significant ($p = 0.06$).

Lower limits of *Fucus spiralis* were closely correlated with the upper limits of *Ascophyllum nodosum*; for the other species, their lower limits were closely correlated with the upper limits of red turf algae (Table VIII). A dramatic rise in the lower limit of fucoids to MWL was evident at Prospect (site 35, Appendix 3). In cases of moderate wave exposure on a steep lower shore (sites 32, 33, 36) *C. crispus* dominated the steeper slopes but *F. evanescens* was co-dominant in the more gently sloping areas. Lower limits of *A. nodosum* were lower in wave-sheltered conditions, while *F. evanescens* extended lower on more gradual slopes (Table VIII) *F. vesiculosus* extended into the sublittoral region at a wave-sheltered site but not at a nearby, semi-exposed site (sites 37-38, Appendix 3).

The heights, relative to tidal levels, of the upper limits of the red-turf species (*Chondrus* and *Mastocarpus*) did not vary significantly between Fundy and Atlantic shores (Fig 5) and were not significantly correlated with any of the variables tested (Table IX); however, the upper limits of dominant (or co-dominant) turfs of these algae were positively correlated with wave exposure and with the lower limits of fucoid algae (Table IX). For kelp species (Fig 7), intertidal upper limits were correlated with slope and geology, with tidal range being not significant (Table IX). Kelps extended farther up on steep shores and on hard substrata; the upper limits were not correlated with lower limits of dominant red turf. The upper limits of dominant stands of intertidal kelp were related to intertidal slope.

A few other species exhibited consistent differences in vertical distribution in the different coastal environments. Species usually restricted to the sublittoral region (*Agarum cribrosum, Callophyllis cristata, Phycodrys rubens* and species of *Phyllophora*) were found in the intertidal region in the Bay of Fundy and, to varying extents, on shore 9 of the Fundy approaches (Table VI; Appendix 2). All of these species were confined below 3 or 5 m depth and became common below 8 to 10 m depth on the Atlantic eastern shore and in the Gulf.

Table VIII Stepwise linear regression modelling of intertidal limits, relative to HHWS, of common fucoid species.*

Species (variables tested)	Type of Limit	(n)	Significant Variable	Effect	Partial R^2	Model R^2	Prob. $>F$
Fucoids	upper	(41)	w	+ve	0.14	0.14	0.01
(tigwhs)			s	+ve	0.09	0.23	0.04
			g	+ve	0.07	0.30	0.06
Fucoids	lower	(16)	D	+ve	0.52	0.52	0.002
(tigwlsDP)			s	+ve	0.14	0.66	0.04
			P	-ve	0.06	0.72	0.14
F. spiralis	upper	(21)	w	+ve	0.26	0.26	0.02
(tigwhs)			s	+ve	0.26	0.52	0.006
F. spiralis	lower	(21)	A	+ve	0.75	0.75	0.0001
(tigwhsA)			w	+ve	0.05	0.79	0.06
A. nodosum	upper	(40)	s	+ve	0.09	0.09	0.06
(tigwhs)							
A. nodosum	lower	(40)	w	+ve	0.11	0.11	0.04
(tigwls)			s	+ve	0.05	0.16	0.13
A. nodosum	lower	(33)	D	+ve	0.55	0.55	0.0001
(tigwlsD)			t	-ve	0.04	0.59	0.09
F. vesiculosus	upper	(41)	s	+ve	0.19	0.19	0.005
(tigwhs)			w	+ve	0.09	0.28	0.03
F. vesiculosus	lower	(39)	D	+ve	0.29	0.29	0.002
(tigwlsD)							
F. evanescens	upper	(30)	s	+ve	0.16	0.16	0.03
(tigwls)			w	+ve	0.15	0.31	0.02
F. evanescens	lower	(21)	s	+ve	0.32	0.32	0.007
(tigwls)							
F. evanescens	lower	(15)	D	+ve	0.64	0.64	0.0004
(tigwlsD)			s	+ve	0.07	0.71	0.11

* Abbreviations: A = upper limit of *A. nodosum;* D = upper limit of dominant *Chondrus* and *Mastocarpus;* P = upper limit of dominant *Laminaria, Alaria,* and *Sacchoriza*.

All other abbreviations given in Table VII.

Table IX Stepwise linear regression modelling of intertidal limits (relative to HHWS) of common macroalgae.*

Species (variables tested)	Type of Limit (n)		Significant Variable	Effect	Partial R^2	Model R^2	Prob. > F
Red turf	upper	(39)	X	+ve	0.09	0.09	0.07
(tigwlsX)			t	-ve	0.06	0.15	0.12
Dominant Red turf	upper	(34)	w	+ve	0.16	0.16	0.02
(tigwls)			s	+ve	0.07	0.22	0.11
Dominant Red turf	upper	(29)	F	+ve	0.38	0.38	0.0003
(tigwlsF)			t	-ve	0.08	0.46	0.06
Kelp	upper	(34)	s	+ve	0.12	0.12	0.04
(tigws)			w	+ve	0.07	0.19	0.11
			t	-ve	0.09	0.28	0.06
			g	+ve	0.09	0.37	0.05
Kelp	upper	(19)	M	+ve	0.15	0.15	0.10
(tigwsM)							
Dominant Kelp	upper	(14)	s	+ve	0.56	0.56	0.002
(tigwsM)			g	+ve	0.10	0.66	0.10

* Abbreviations: F = lower limit of Fucoids; M = lower limit of dominant Red turf; X = lower limit of dominant fucoids.

All other abbreviations given in Tables VII & VIII.

Many common species, including *Cystoclonium purpureum, Ectocarpus siliculosus, Petalonia fascia, Ulva lactuca, Ulvaria oxysperma, Palmaria palmata, Devaleraea ramentacea,* and *Dumontia contorta* were found in the intertidal region at all wave exposures but extended farther up the shore in the more wave-exposed sites (Appendix 2). *Alaria esculenta* dominated the sublittoral fringe only in semi-exposed and exposed conditions, with the exception of Mascabin Point (site 13), where there is a swift tidal current. *A. esculenta* was often present but not dominant on semi-sheltered shores and absent from sheltered shores.

Patterns of distribution in the sublittoral region. Sublittoral vegetation was extremely patchy and variable (Appendix 3). At all sites and depths, hard substrata were occupied by encrusting coralline algae. The canopy of wave-exposed, Atlantic and Fundy sites was often dominated by 90-100% cover by species of *Laminaria* and *Desmarestia.*

Where the kelp canopy was dense, the understory often consisted of scattered *Chondrus crispus* and *Corallina officinalis,* whereas under more open canopies species of *Polysiphonia* and *Rhodomela confervoides* were co-dominant. On Atlantic shores *Dumontia contorta, Devaleraea ramentacea* and *Cystoclonium purpureum* were common in the understory down to 6 m depth; *Phycodrys rubens, Callophyllis cristata, Ptilota serrata* and, occasionally, species of *Phyllophora, Odonthalia dentata* and *Neodilsea integra* provided the understory in deeper water (Appendix 3).

In sheltered sites where sea urchin herbivory had recently ceased or been drastically reduced (sites 34a, 37, Appendix 3), species of *Laminaria* were either rare or did not form dense stands, and a variety of smaller red and brown algae were co-dominant. Laminarians were also rare where the bottom was unstable (site 44, Appendix 3).

When many sea urchins were present in the sublittoral region (Fig 7) *Laminaria* was usually rare, particularly at depths greater than 2-4 m. The vegetation could be reduced to crustose corallines, or consist mainly of *Desmarestia, Agarum* and scattered individuals of *Phyllophora, Chondrus* and *Polysiphonia* (sites, 3, 11, 40-42, Appendix 3).

The upper and lower boundaries of *Laminaria* varied both among and within the coastal environments (Fig 7, Table VI). Where kelp was confined to the sublittoral region, the upper limit was correlated with ice scour (Table X). A friable substratum such as occurs in the Gulf sites may exacerbate the effect of ice scour. However, kelp occurred intertidally on sandstone and shale in the Bay of Fundy (sites 7, 17), suggesting that a friable substrate does not by itself prevent the occurrence of macroalgae in wave-swept environments.

On the ice-scoured sites of Cape Breton Island (sites 42, 44, Appendix 3), as in the Gulf (sites 65-69, Appendix 3), fucoids, *Chondrus crispus,* or small ephemeral brown algae replaced *Laminaria* in shallow water. These gave way, at 4-8 m depth, to *C. crispus* or *Desmarestia, Rhodemela confervoides* and species of *Polysiphonia* and *Phyllophora.* On shore 17 of the Gulf (sites 65-83, Appendix 3) and also at some north-shore sites (McLachlan *et al.,* 1987), a shallow-water belt of fucoids and *Chondrus crispus* often gave way to *Furcellaria lumbricalis* to about 10 m and then to species of *Phyllophora* to about 20 m. On these and other Gulf shores, mussels could dominate extensive areas (site 83, Appendix 3). *Laminaria* was rare down to 3-6 m. Individual kelp plants were small (< 1 m long) compared to plants of Atlantic and Fundy shores, and cover by the canopy rarely exceeded 60%.

Lower limits of species of *Laminaria* were correlated with tidal range, sublittoral sand, sea urchin activity and ice scour (Table X). The lower limits of *Laminaria,* and of foliose algae in general, were much shallower in the macrotidal Bay of Fundy than on the Atlantic coast and in the Gulf (Tables VI, XI). The lower limit of significant foliose

Table X Stepwise linear regression modelling of sublittorally occurring limits of common macroalgae.*

Species (variables tested)	Type of Limit (n)	Significant Variable	Effect	Partial R^2	Model R^2	Prob. > F
Fucoids	lower (51)	i	-ve	0.49	0.49	0.0001
(tigwbuR)		b	-ve	0.03	0.52	0.08
		R	+ve	0.04	0.57	0.03
		g	+ve	0.02	0.59	0.13
		t	-ve	0.02	0.61	0.15
Red Turf	lower (69)	i	-ve	0.50	0.50	0.0001
(tigwbulK)		K	+ve	0.02	0.52	0.06
Kelp	upper (50)	i	-ve	0.10	0.10	0.03
(tigwbu)		u	+ve	0.05	0.15	0.11
Laminaria	lower (69)	t	+ve	0.63	0.63	0.0001
(tigwbu)		b	+ve	0.09	0.72	0.0001
		u	+ve	0.02	0.75	0.02
		i	-ve	0.02	0.77	0.02
		w	-ve	0.01	0.78	0.12

* Abbreviations given in Table VII.

Table XI Vertical position and nature of the lower limits of significant foliose algal vegetation in each coastal environment.*

	Total Sites (n)	No. of Sites with Algal Limits in Each Depth Range			Nature & Depth of Lower Limits in 0-20 m Depth Range					
					No. of each type				Depth (m)	
		Int	0-20 m	> 20 m	nd	Sand	Urch	Rock	SE	
Fundy shores										
1-7	21	4	11	0	6	6	5	4	7	1.2
Atlantic shores										
8-9	9	1	1	1	6	1	0	1	10	
10-13	13	0	11	1	1	11	0	0	11	1.0
14-15	3	0	3	0	0	2	1	0	8	1.2
Gulf shores										
17-20	39	0	35	4	0	6	15	14	16	0.4
21-22	5	0	3	2	0	3	0	0	11	0

* Abbreviations: Int = intertidal, Urch = aggregation of sea urchins, Sand = sand or cobble, Rock = bedrock or boulders, nd = no data.

vegetation could occur on shallow, bare rock in the Bay of Fundy and Fundy approaches. Limits on the outer Atlantic coast and in the Gulf only occurred on rock at depths greater than 15 m; otherwise they were defined by unstable bottom or aggregations of sea urchins (Table XI). The correlation between maximum depth of *Laminaria* and ice scour results from the occurrence of deep rock flats at many of the Gulf sites.

Ice scour and the upper limits of red turf were significantly correlated with lower fucoid limits in the sublittoral region (Table X); fucoids extended deeper on disturbed sites, and the lower limits of *Chondrus* were similarly found lower down at ice-scoured sites (Table X). The relationship between the lower limits of red turf and the upper limits of kelp was insignificant.

Where suitable hard substratum was available on Fundy and Atlantic shores, there was a deep-water zone occupied by *Agarum cribrosum* and various red algae which lay either below or overlapping the lower portion of the *Laminaria-Desmarestia* belt. The upper limit of *Agarum* was intertidal or to 8 m depth in the Bay of Fundy and at 2-12 m on the Atlantic coast (Fig 7, Table VI). In the Gulf *A. cribrosum* occurred at depths $>$ 12 m and was sometimes absent from depths $<$ 20 m.

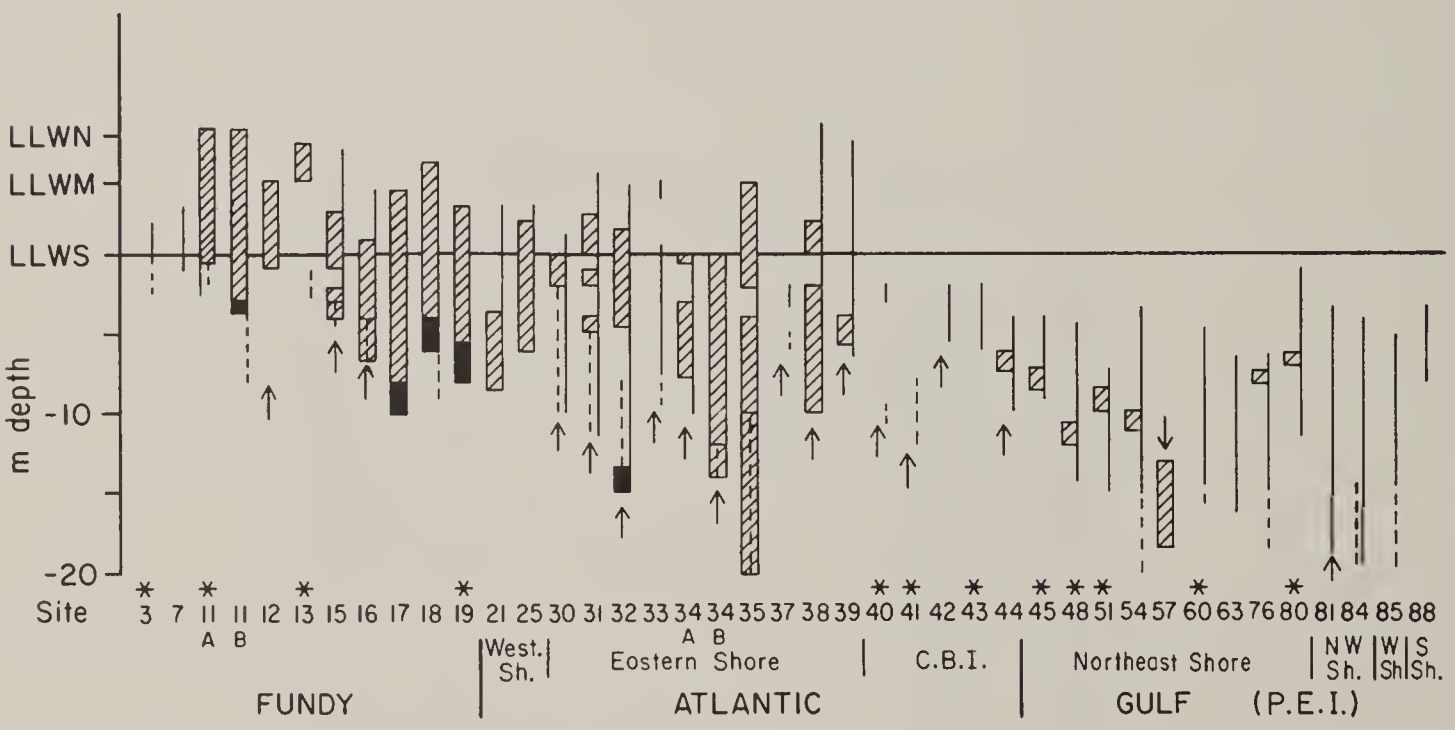

Fig 7 Limits of kelp species in all sublittoral surveys of Fundy and Atlantic coasts and at selected Gulf sites. Thick bars indicate dominance; thin lines, presence. *Alaria* or *Laminaria* = diagonal bar and solid line; *Agarum* = solid bar and dashed line. Other symbols as in Fig 5.

Discussion

The intertidal regions (between HHWS and LLWS) of stable, rocky shores of the north-eastern Atlantic Ocean generally exhibit three vegetational zones: the littoral fringe, the midlittoral zone, and the sublittoral fringe (Stephenson and Stephenson, 1954; Wilce, 1959; Bolton, 1981). Data from our survey support this generalization, but it is obvious that on these shores, differences in tidal amplitude (Fig 8a-b), slope and wave exposure (Fig 8c), ice scour (Fig 8d) and, to a lesser extent, geology affect not only the vertical distance occupied by the various zones but also their positions relative to tidal levels and their species compositions.

Physical factors may limit the vertical extent of intertidal plants by exceeding limits of tolerance of individual species or by affecting competitive balances among species

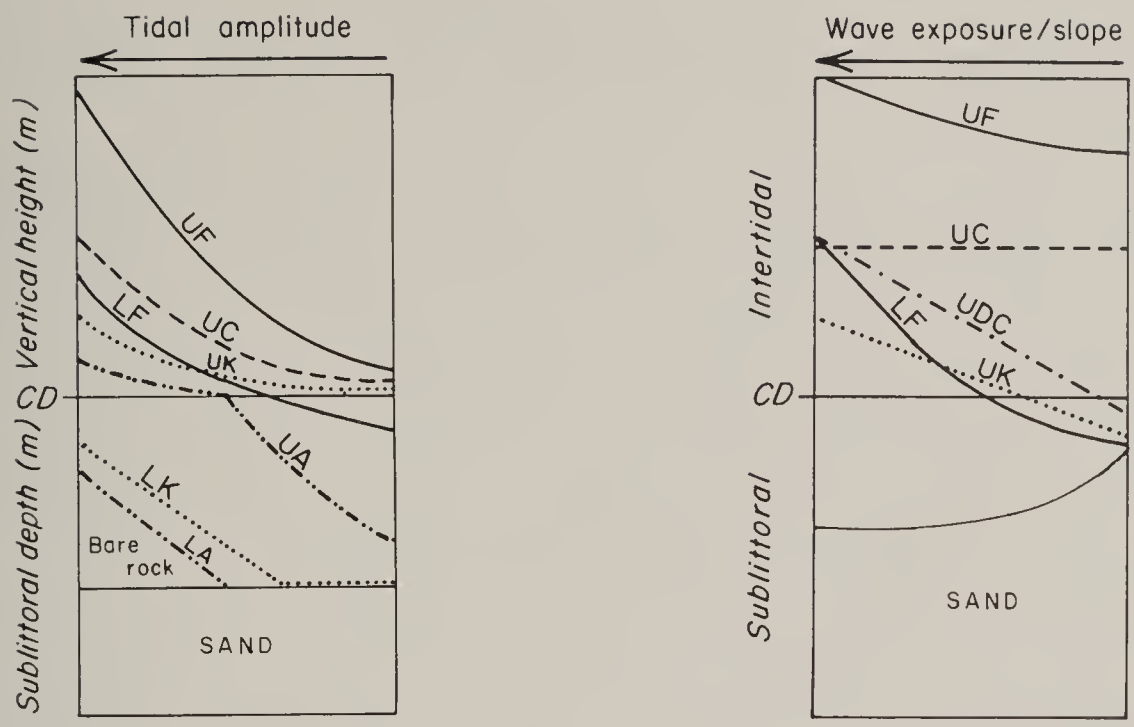

Fig 8 Schematic representations of general patterns of vertical distribution of macroalgae on rocky shores of the Maritime Provinces, Canada. a-b) Changes in heights of algal limits with tidal amplitude; a) absolute heights [m]; b) relative heights (% HHWS). c) Changes in relative heights with slope and wave exposure. d) Changes in relative heights with presence and absence of ice scour. UF/LF = upper/lower limit of fucoids; UC = upper limit of *Chondrus crispus* and *Mastocarpus stellatus;* UDC = upper limit of dominant red turfs; UK/LK = upper/lower limits of kelp species, except for *Agarum cribrosum;* UA/LA = upper/-lower limits of *A. cribrosum.*

(Widdowson, 1965; Kussakin, 1977; Southward and Southward, 1978; Lubchenco 1980; Druehl and Green, 1982). When physical factors are not limiting, the lower and upper boundaries of intertidal and sublittoral algae are largely determined by competition and herbivory (Menge, 1976; Lubchenco and Menge, 1978; Lubchenco, 1980; Underwood and Denley, 1984).

We have found that, as is the case on European shores (Lewis, 1964), upper distributional limits of many intertidal species in eastern Canada are higher on relatively steep, wave-exposed shores (Fig 8c). The relatively depressed upper limits of fucoids along the Fundy approaches (Table V) therefore appear to be related to the fact that all the sites surveyed on these shores were either semi-sheltered or gently sloping (Appendix 1). These upper limits may therefore be influenced by desiccation but experimental tests are needed to determine the effect of herbivory. Wave exposure also influences species composition. Moving from sheltered to steep and/or wave-exposed sites, species of *Fucus, Mastocarpus* and *Alaria* replaced *Ascophyllum, Chondrus* and *Laminaria,* respectively.

The extension of sublittoral algae and certain invertebrates (Bousfield and Laubitz, 1972) into the intertidal region of the Bay of Fundy is readily explained in terms of decreased illuminance and desiccation in this environment. A similar upward extension of sublittoral species has been documented in foggy and wave-swept environments such as the Faeroes (Price and Farnham, 1982) and the northern shores of Newfoundland and Labrador (Wilce, 1959).

For fucoid species, the availability of stable and resistant substrata in the upper intertidal region affects upper limits. Similarly, kelp species extend higher in the intertidal on resistant bedrock than on soft rock. The upper limits of all perennial macroalgae in the Gulf and on some Atlantic shores are depressed owing to winter ice scour. On such disturbed substrata ephemerals dominate; perennial fucoids occur sublittorally here, as they do on ice-scoured shores of Newfoundland and Labrador (Wilce, 1959). Where ice scour is relatively minor and rocky substrata are available, the upper limit of fucoids in the Gulf is still depressed (Lobban and Hanic, 1984), probably because of desiccation stress. This coastal environment is characterized by relatively high numbers of sunshine hours and infrequent fog, together with mixed, semi-diurnal tides that sometimes leave the intertidal region exposed for extended periods during day time in summer.

Little is known of competitive interactions among algal species. However, the vertical arrangement of intertidal fucoid species in eastern Canada is similar to that in Britain (Lewis, 1964), where the pattern is largely a result of interspecific competition (Burrows and Lodge, 1951). Competition from *Chondrus crispus* can prevent fucoids from occupying otherwise favorable areas of the low intertidal (Lubchenco, 1980).

The lower limit of fucoids bordered on dominant stands of *Chondrus crispus* and *Mastocarpus stellatus* and these, in turn, frequently bordered on dominant stands of kelp. However, the shore level of the boundaries between fucoids, red turf algae and kelp varied among the coastal environments. Our statistical analyses indicate that, depending upon the species involved, the variations were related to tidal range, intertidal slope, wave exposure and disturbance (Fig 8). These physical factors may, therefore, be important to the outcome of competitive interactions.

In the Bay of Fundy *Chondrus, Mastocarpus* and patches of other small algae, either alone or with kelps, usually excluded fucoids below LLWM. In contrast, on the Atlantic coast, particularly at wave-sheltered sites, fucoids usually extended down below LLWS. Only at one steep and wave-exposed site (site 35) were fucoids entirely excluded from the lower intertidal region of an Atlantic shore.

The features that set the Bay of Fundy apart from the Atlantic and Gulf environments are the extreme tidal range and relatively steep intertidal slopes, factors that are

both significantly and positively correlated with the lower limits of the fucoid belt. There are several possible explanations for this correlation.

Daily quantum dose declines steadily from the top to the bottom of any shore and this reduction is greatest where tides are highest (Dring, 1987). In the British Isles, lack of light has been invoked to explain the limited downward extension of fucoids in turbid and macrotidal waters (Gail, 1918; Burrows and Lodge, 1951; Dring, 1987). Lack of light may similarly be the factor restricting the distribution of fucoids in the Bay of Fundy, as the frequent occurrence of spring low tides in the morning and evening, the turbidity of the water, and the large tidal range all limit the light reaching the lower intertidal region. In contrast to fucoids, *Chondrus crispus* has a lower light saturation point (Mathieson and Burns, 1971; Burns and Mathieson, 1972; Neimeck and Mathieson, 1978; Bird *et al.*, 1979). An ability to grow in limited light, together with the fact that the timing of low tides and frequency of fog decrease desiccation, may explain *Chondrus* as a competitive dominant this environment.

Where the tidal range is great, the low intertidal region is subject to long periods of intense tidal currents as well as surface-wave activity (Thomas *et al.*, 1983). The physical stresses of currents and waves are most pronounced on steeply sloping shores. Such mechanical stress may make the low intertidal zone of steep Fundy shores less suitable for larger macrophytes than for tenacious turf forms, and may particularly favor *Mastocarpus stellatus.* Foliose *M. stellatus* is abundant in the Bay of Fundy, occasionally present on steep or wave-exposed shores of the Atlantic coast and absent from the Gulf. Like *Chondrus crispus, M. stellatus* is intolerant of desiccation (Marshall *et al.*, 1949) but it is more prevalent than *C. crispus* on wave-exposed or current-stressed coasts (Marshall *et al.*, 1949; Mathieson *et al.*, 1977). The cool summer temperatures of the Bay of Fundy may also favor *M. stellatus* over *C. crispus* (Burns and Mathieson, 1972; Munda, 1977; Guiry and West, 1983).

When kelps are rare or absent because of disturbance from ice or sand scour, fucoids can form dominant stands to 6-8 m depth. Moderate disturbance can increase diversity (Dayton, 1971) and may facilitate the recruitment of fucoids among otherwise dominant *Chondrus crispus* (MacFarlane, 1952), at least where light is not limiting. The lower limit of fucoids may then be set by dense aggregations of mussels or sea urchins, as is common in Northumberland Strait (Moseley and MacFarlane, 1969), or by competition from species that grow better at low light levels. *Rhodomela confervoides* and species of *Polysiphonia* seem able to out-compete fucoids in the sublittoral region of sheltered Atlantic shores. Dense stands of *Furcellaria lumbricalis* or *Chondrus crispus* often confine the sublittoral fucoid belt in the Gulf. The lower limit of *C. crispus* in turn tends to be deeper at these disturbed sites, perhaps because of the lack of effective competition from kelps. Field experiments are needed to clarify the roles of competition, herbivory and disturbance in this environment.

Three belts of vegetation have been recognized in the sublittoral region of rocky Atlantic shores (Edelstein et al., 1969): a *Laminaria-Desmarestia* belt to about 15 m, an *Agarum-Ptilota* belt below 10 m, and, below 30 m, a *Phyllophora-Polysiphonia* belt. Other workers have described other dominant assemblages, mostly in shallow water on unstable substrata (Moseley and MacFarlane, 1969; Mann, 1972; McPeak, 1980). Our data confirm the existence of a *Laminaria-Desmarestia* belt along most of the rocky Atlantic and Fundy coasts, but variations occur that are related to the availability of firm substrata and the activity of herbivorous sea urchins. Dominance by sea urchins or by kelp can be viewed as opposite ends of the dynamic continuum that is the "*Laminaria-Desmarestia* zone" (Schiel and Foster, 1986). At some Atlantic sites, sea urchins had recently died and *Desmarestia* was dominant. Such sites were probably in transition, and will in time become dominated by *Laminaria* (Johnson, 1984). In wave-sheltered sites formerly occupied by sea urchins, establishment of

Laminaria and *Desmarestia* was more erratic, possibly because these sites lack a wave-battered sublittoral fringe which can provide a refuge from sea urchin herbivory and therefore a persistent spore source. The scarcity of *Odonthalia dentata* and species of *Phyllophora* on the eastern Atlantic shore where they were once common (Edelstein *et al.*, 1969; Novaczek and McLachlan, 1986) may be due to lack of such deep-water species in shallow-water refugia.

In the Gulf the sublittoral region, with its frequent patches of sand and cobble, was particularly variable. Different vegetation belts replaced the *Laminaria-Desmarestia* zone at different depths and on different substrata. Both in the Gulf and along northeastern Cape Breton Island, the rarity and small size of *Laminaria* could result from the friable substratum, ice scour, sea urchin herbivory and, perhaps, competition from red-algal turfs. Recruitment of *Laminaria* in the sublittoral region can be impeded by dense turfs of red algae (Chapman, 1984). Again, experimental work is required to clarify the roles of various factors.

The *Agarum-Ptilota* belt (Edelstein *et al.*, 1969) may occur at less than 20 m depth in the Gulf but may also be lacking or confined to deeper water (Bird *et al.*, 1983). We found a similar *Agarum-Ptilota* belt in shallower water (<10 m) below the *Laminaria-Desmarestia* belt on Atlantic and Fundy shores. Because of the depth limits of our survey, we cannot comment on the general occurrence of a deep *Phyllophora-Polysiphonia* assemblage on Atlantic and Gulf shores. In the Bay of Fundy this vegetation belt is lacking. Even on hard substrata and where sea urchins were not observed, the lower limits of foliose algal growth in the Bay of Fundy were shallow and sublittoral algal cover was sparse compared to other coastal environments. The reasons for this, which need to be studied experimentally, may include turbidity (Logan *et al.*, 1983; Prouse, 1983), herbivory, and tidal currents.

Variation in the complement of species found in the coastal environments may stem from historical as well as environmental influences. For instance, *Furcellaria lumbricalis* and *Fucus serratus* were previously restricted to the Gulf. This pattern may reflect the locations where these species were introduced, presumably from Europe. Their limited success in spreading out of the Gulf may be related to physical or biological factors (Edelstein *et al.*, 1972; Dale, 1982; Holmsgaard *et al.*, 1981), although *Fucus serratus* has now become relatively abundant and generally wide spread along the Fundy approaches. Gulf populations, especially those in shallow embayments, of warm-temperate species appear to be relics of a time 7000 years ago when the Atlantic coastal waters were several degrees warmer (Novaczek *et al.*, 1987).

The rarity of *Ascophyllum nodosum* in the Gulf is probably owing to the combination of friable substratum and ice scour (Bird *et al.*, 1983). The absence of *Alaria esculenta* may be primarily a consequence of lethal high summer temperatures (Sundene, 1962) but ice scour (Keats *et al.*, 1985) is also a possible factor.

Other cases of absence of species from coastal environments appear to be related to the summer sea temperature, with southern species often being restricted to the Gulf and northern species restricted to the colder Fundy and Atlantic environments. The number of warm-temperate species and the tendency for normally intertidal algae to occur sublittorally in the Gulf made the sublittoral region of this environment particularly rich in species (Fig 4). The absence of Arctic species from the depauperate sublittoral region of the Bay of Fundy may in some cases be related to the moderate minimum winter temperatures together with limited availability of substratum.

Patterns of nutrient availability can direct the evolution of physiological ecotypes and affect the phenology of an algal species (Espinosa and Chapman, 1983). There is also evidence that on enriched shores of the Bay of Fundy and Fundy approaches both individual plant size and biomass per unit area can be large (MacFarlane, 1952; McPeak, 1980; Pringle and Semple, 1980) relative to that on Atlantic shores (Mann, 1972; Cousens, 1981; Scheibling, 1986) and Gulf shores (Herring and MacBeth, 1973;

McLachlan *et al.*, 1987). Four of the species we recorded were restricted to nutrient-enriched shores of the Bay of Fundy and Fundy approaches. However, these were either small or taxonomically difficult and may have been overlooked at other sites. The fact that the number of species recorded for the Atlantic coast increased from 168 to 177 with the addition of data from the Fundy approaches may indicate an increase in diversity related to nutrient enrichment.

Our survey has documented a wealth of variation in the vertical distributions of algal species on rocky shores of the Maritime provinces. There are, however, general trends in the distributions of dominant species (Figs 4-8), which can be attributed to major differences in physical factors among the three coastal environments. From the Bay of Fundy around the Atlantic coast and into the southern Gulf of St. Lawrence, the tidal characteristics, the type of substratum, the slope of the intertidal region and the degree of winter ice scour all change; in the same progression, the belts of dominant algae (fucoids, *Chondrus-Mastocarpus, Laminaria-Desmarestia* and *Agarum-Ptilota*) occupy positions farther and farther down the shore. Before we can fully understand the variations within this general pattern, experimental studies of biotic interactions will be necessary. Our analyses suggest that any such experimental program should incorporate controls to test for the influence of variations in tidal range, slope, wave exposure and disturbance on biotic interactions.

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References

Adey, W.H. 1964. The genus *Phymatolithon* in the Gulf of Maine. *Hydrobiologia* 24: 377-420.

Adey, W.H. 1965. The genus *Clathromorphum* (Corallinaceae) in the Gulf of Maine. *Hydrobiologia* 26: 538-573.

Bailey, W.B. 1954. Seasonal variations in the hydrographic conditions of the Bay of Fundy. *Fish. Res. Board Can.* Manus. Rept. Biol. Stn. No. 551.

Bell, H.P. and **MacFarlane, C.** 1933a. The marine algae of the Maritime provinces of Canada. I. List of species with their distribution and prevalence. *Can. J. Res.* 9: 265-279.

Bell, H.P. and **MacFarlane, C.** 1933b. The marine algae of the Maritime provinces of Canada. II. A study of their ecology. *Can. J. Res.* 9: 280-293.

Bird, C.J., Greenwell, M. and **McLachlan, J.** 1983. Benthic marine algal flora of the north shore of Prince Edward Island (Gulf of St. Lawrence) Canada. *Aquat. Bot.* 16: 315-335.

Bird, N.L., Chen, L.C.-M. and **McLachlan, J.** 1979. Effects of temperature, light and salinity on growth in culture of *Chondrus crispus, Furcellaria lumbricalis, Gracilaria tikvahiae* (Gigartinales, Rhodophyta) and *Fucus serratus* (Fucales, Phaeophyta). *Bot. Mar.* 22: 521-527.

Blair, S.M. 1983. Taxonomic treatment of the *Chaetomorpha* and *Rhizoclonium* species (Cladophorales: Chlorophyta) in New England. *Rhodora* 85: 175-211.

Bolton, J.J. 1981. Community analysis of vertical zonation patterns on Newfoundland rocky shores. *Aquat. Bot.* 10: 299-316.

Bousfield, E.L. and **Laubitz, D.R.** 1972. Station lists and new distributional records of littoral marine invertebrates of the Canadian Atlantic and New England regions. *Nat. Mus. Nat. Sci. (Ottawa) Publ. Biol. Ocean.* 5: 1-51.

Burns, R.L. and **Mathieson, A.C.** 1972. Ecological studies of economic red algae. II. Culture studies of *Chondrus crispus* and *Gigartina stellata*. *J. Exp. Mar. Biol. Ecol.* 8: 1-6.

Burrows, E.M., and **Lodge, S.** 1951. Autecology and the species problems in *Fucus*. *J. Mar. Biol. Assoc. U.K.* 30: 161-176.

Canadian Climate Normals. 1982. Vol. 7. *Bright sunshine*. Canadian Climate Program, Environment Canada.

Canadian Climate Normals. 1984. Vol. 9. *Soil temperature, lake evaporation, days with blowing snow, hail, fog, smoke/haze, frost.* Canadian Climate Program, Environment Canada.

Canadian Tide and Current Tables, 1984. Vols. 1, 2. Government of Canada, Fisheries and Oceans.

Cardinal, A. 1968. Répertoire des algues marines benthiques de l'est du Canada. *Cahiers d'Information 48.* Ministère de l'Industrie et du Commerce, Québec.

Chamberlain, Y.M. 1983. Studies in the Corallinaceae with special reference to *Fosliella* and *Pneophyllum* in the British Isles. *Bull. Br. Mus. (Nat. Hist.) Bot.* 11: 291-463.

Chapman, A.R.O. 1981. Stability of sea urchin dominated barren grounds following destructive grazing of kelps in St. Margaret's Bay, eastern Canada. *Mar. Biol.* 62: 307-311.

Chapman, A.R.O. 1984. Reproduction, recruitment and mortality in two species of *Laminaria* in southwest Nova Scotia. *J. Exp. Mar. Biol. Ecol.* 78: 99-109.

Chapman, A.R.O. and **Craigie, J.** 1977. Seasonal growth in *Laminaria longicruris:* Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* 40: 197-205.

Chapman, A.R.O. and **Gagné, J.** 1980. Environmental control of kelp growth in St. Margaret's Bay and on the southwest shore of Nova Scotia. *Can. Tech. Rept. Fish. Aquat. Sci.* 954: 194-207.

Colinvaux, L.H. 1966. Distribution of marine algae in the Bay of Fundy, New Brunswick, Canada. *Proc. Int. Seaweed Symp.* 5: 91-98.

Colinvaux, L.H. 1970. Marine algae of eastern Canada: A seasonal study in the Bay of Fundy. *Nova Hedwigia* 19: 139-158.

Coote, A.R. and **Hiltz, R.S.** 1975. Distribution of silicate, nitrate and phosphate in the Gulf of St. Lawrence: July-August 1971, June 1972 and February 1975. *Bedford Inst. Oceanogr. Rept. Serv.* BI-R-75-14, Dartmouth, N.S.

Coote, A.R. and **Yeats, P.A.** 1979. Distribution of nutrients in the Gulf of St. Lawrence. *J. Fish. Res. Board Can.* 36: 122-131.

Cousens, R. 1981. *The population biology of Ascophyllum nodosum (L.) Le Jolis.* Ph.D. thesis, Dalhousie University, Halifax, N.S.

Dale, M. 1982. Phytosociological structure of seaweed communities and the invasion of *Fucus serratus* in Nova Scotia. *Can. J. Bot.* 60: 2652-2658.

Dayton, P.K. 1971. Competition, disturbance and community organization: The provision and subsequent utilization of space in a rocky intertidal community. *Ecol. Monogr.* 41: 351-389.

Dobson, D. and **Petrie, B.** 1982. Long-term temperature monitoring program 1981. *Can. Data Rept. Hydrogr. Ocean Sci.* 6: 1-297.

Dring, M.J. 1987. Light climate in intertidal and subtidal zones in relation to synthesis and growth of benthic algae: a theoretical model. In: *Plant life in aquatic and amphibious habitats.* (Ed. R.M. Crawford). Special Publications No. 5, Br. Ecol. Soc., Blackwell Scientific Publ. Oxford, pp. 359-373.

Drinkwater, K. and **Taylor, G.** 1982. Monthly means of the temperature, salinity and density along the Halifax section. *Can. Tech. Rep. Fish. Aquat. Sci.* 1093: 1-67.

Druehl, L.D., and **Green, J.M.** 1982. Vertical distribution of intertidal seaweeds as related to patterns of submersion and emersion. *Mar. Ecol. Prog. Ser.* 9: 163-170.

Edelstein, T., Craigie, J.S. and **McLachlan, J.** 1969. Preliminary survey of the sublittoral flora of Halifax County. *J. Fish. Res. Board Can.* 26: 2703-2713.

Edelstein, T., Greenwell, M., Bird, C.J. and **McLachlan, J.** 1972. Investigations of the marine algae of Nova Scotia X. Distribution of *Fucus serratus* L. and some other species of *Fucus* in the Maritime Provinces. *Proc. N.S. Inst. Sci.* 27: 33-42.

El-Sabh, M.I. 1976. Surface circulation pattern in the Gulf of St. Lawrence. *J. Fish. Res. Board Can.* 33: 124-138.

Espinosa, J. and **Chapman, A.R.O.** 1983. Ecotypic differentiation of *Laminaria longicruris* in relation to seawater nitrate concentration. *Mar. Biol.* 74: 213-218.

Gagné, J.A. and **Mann, K.H.** 1981. Comparison of growth strategy in *Laminaria* populations living under different seasonal patterns of nutrient availability. *Proc. Int. Seaweed Symp.* 10: 297-302.

Gail, F.W. 1918. Some experiments with *Fucus* to determne the factors controlling its vertical distribution. *Puget Sound Mar. Biol. Stn. Publ.* 2: 139-151.

Garret, C.J.R. and **Loucks, R.A.** 1976. Upwelling along the Yarmouth shore of Nova Scotia. *J. Fish Res. Board Can.* 33: 116-117.

Gran, H.H. and **Braarud, T.** 1935. A quantitative study of the phytoplankton in the Bay of Fundy and the Gulf of Maine (including observations on hydrography, chemistry and turbidity). *J. Biol. Board Can.* 1: 279-467.

Guiry, M.D. and **West, J.A.** 1983. Life history and hybridization studies on *Gigartina stellata* and *Petrocelis cruenta* (Rhodophyta) in the North Atlantic. *J. Phycol.* 19: 474-494.

Guiry, M.D., West, J.A., Kim, D.-H. and **Masuda, M.** 1984. Reinstatement of the genus *Mastocarpus* Kützing (Rhodophyta). *Taxon* 33: 53-63.

Hanic, L.A. 1974. *A guide to the common seaweeds of Prince Edward Island.* P.E.I. Mar. Sci. Club Publ., Charlottetown.

Hartnoll, R.G. and **Hawkins, S.J.** 1980. Monitoring rocky-shore communities: A critical look at spatial and temporal variation. *Helgol. Meeresunters* 33: 484-494.

Herring, F. and **MacBeth, C.** 1973. *Quantity survey of Chondrus crispus and other marine plants, Covehead to Naufrage, Prince Edward Island.* P.E.I. Dept. Fish. Tech. Rep. 130, Charlottetown, P.E.I.

Himmelman, J.H. 1980. The role of the green sea urchin, *Strongylocentrotus droebachiensis,* in the rocky subtidal region of Newfoundland. *Can. Tech. Rept. Fish. Aquat. Sci.* 954: 92-119.

Holmsgaard, J.E., Greenwell, M. and **McLachlan, J.** 1981. Biomass and vertical distribution of *Furcellaria lumbricalis* and associated algae. *Proc. Int. Seaweed Symp.* 10: 309-313.

Johnson, C.R. 1984. *Ecology of the kelp Laminaria longicruris and its principal grazers in the rocky subtidal of Nova Scotia.* Ph.D. thesis, Dalhousie University, Halifax, N.S.

Johnson, C.R. and **Mann, K.H.** 1986. The importance of plant defence abilities to the structure of subtidal seaweed communities: The kelp *Laminaria longicruris* de la Pylaie survives grazing by the snail *Lacuna vincta* (Montagu) at high population densities. *J. Exp. Mar. Biol. Ecol.* 97: 231-267.

Keats, D.W., South, G.R. and **Steele, D.H.** 1985. Algal biomass and diversity in the upper subtidal at a pack-ice disturbed site in eastern Newfoundland. *Mar. Ecol. Prog. Ser.* 25: 151-158.

Kussakin, O.G. 1977. Intertidal ecosystems of the seas of the USSR. *Helgol. Wiss. Meeresunters.* 30: 243-262.

Lauzier, L.M. 1967. Bottom residual drift on the continental shelf area of the Canadian Atlantic coast. *J. Fish. Res. Board Can.* 24: 1845-1858.

Lauzier, L.M. and **Hull, J.H.** 1969. Coastal station data temperatures along the Canadian Atlantic coast, 1921-1969. *Fish. Res. Board Canada Tech. Rept.* 150: 1-25.

Lewis, J.R. 1964. *The ecology of rocky shores.* English University Press Ltd., London.

Lindstrom, S.C. 1985. Nomenclatural and taxonomic studies on *Dilsea* and *Neodilsea* (Dumontiaceae, Rhodophyta). *Taxon* 34: 260-266.

Lobban, C.S., and **Hanic, L.A.** 1984. Rocky shore zonation at North Rustico and Prim Point, Prince Edward Island. *Proc. N.S. Inst. Sci.* 34: 25-40.

Logan, A., MacKay, A.A. and **Noble, J.P.A.** 1983. *Chapter 8,* Sublittoral hard substrates. In: *Marine and coastal systems of the Quoddy Region, New Brunswick.* (ed. M.L.H. Thomas). *Can. Spec. Publ. Fish. Aquat. Sci.* 64: 119-139.

Loring, D.H. and **Nota, D.J.G.** 1973. Morphology and sediments of the Gulf of St. Lawrence. *Fish. Mar. Serv. Bull.* 182: 1-147.

Lubchenco, J. 1980. Algal zonation in the New England rocky intertidal community: An experimental analysis. *Ecology* 61: 333-344.

Lubchenco, J. 1983. *Littorina* and *Fucus*: effects of herbivores, substratum heterogeneity and plant escapes during succession. *Ecology* 64: 1116-1123.

Lubchenco, J. and **Menge, B.A.** 1978. Community development and persistence in a low rocky intertidal zone. *Ecol. Monogr.* 59: 67-94.

MacFarlane, C.I. 1952. A survey of certain seaweeds of commercial importance in southwest Nova Scotia. *Can. J. Bot.* 30: 78-97.

MacFarlane, C.I. 1965. Sublittoral surveying for commercial seaweeds in Northumberland Strait. *Proc. Int. Seaweed Symp.* 5: 169-176.

Mann, K.H. 1972. Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada: I. Zonation and biomass of seaweeds. *Mar. Biol.* 12: 1-10.

Marine Ecology Laboratory. 1980. Physical oceanography, dissolved nutrients, phytoplankton production, plankton biomass and sedimentation in St. Georges Bay, N.S., 1977. *Can. Tech. Rept. Fish. Aquat. Sci.* 934: 1-162.

Marshall, S.M., Newton, L. and **Orr, A.P.** 1949. *A study of certain British seaweeds and their utilisation in the preparation of agar.* H.M. Stationery Office, London.

Mathieson, A.C. and **Burns, R.L.** 1971. Ecological studies of economic red algae: I. Photosynthesis and respiration of *Chondrus crispus* Stackhouse and *Gigartina stellata* (Stackhouse) Batters. *J. Exp. Mar. Biol. Ecol.* 7: 197-206.

Mathieson, A.C., Tveter, E., Daly, M. and **Howard, J.** 1977. Marine algal ecology in a New Hampshire tidal rapid. *Bot. Mar.* 20: 277-290.

MacLachlan, J., Greenwell, M., Bird, C.J. and **Holmsgaard, J.E.** 1987. Standing stocks of seaweeds of commercial importance on the north shore of Prince Edward Island, Canada. *Bot. Mar.* 30: 277-289.

McPeak, R.H. 1980. A preliminary assessment of the *Laminaria* resource near Lower Wood Harbour, Nova Scotia, during July 1977. *Can. Tech. Rept. Fish. Aquat. Sci.* 954: 180-192.

Menge, B.A. 1976. Organization of the New England rocky intertidal community: Role of predation, competition and environmental heterogeneity. *Ecol. Monogr.* 46: 355-393.

Miller, R.J. 1985. Succession in sea urchin and seaweed abundance in Nova Scotia, Canada. *Mar. Biol.* 84: 275-286.

Miller, R.J. and **Colodey, A.G.** 1983. Widespread mass mortalities of the green sea urchin in Nova Scotia, Canada. *Mar. Biol.* 73: 263-267.

Moore, D.S. and **Miller, R.J.** 1983. Recovery of macroalgae following widespread sea urchin mortality with a description of the nearshore hard-bottom habitat on the Atlantic coast of Nova Scotia. *Can. Tech. Rept. Fish. Aquat. Sci.* 1230: 1-94.

Moore, D.S., Miller, R.J. and **Meade, L.D.** 1986. Survey of shallow benthic habitat: Eastern shore and Cape Breton, Nova Scotia. *Can. Tech. Rept. Fish. Aquat. Sci.* 1546: 1-49.

Moseley, C.M. and **MacFarlane, C.I.** 1969. *Sublittoral seaweed investigation in Northumberland Strait and George Bay 1967-1968.* Nova Scotia Research Foundation Corp., Seaweeds Division, Dartmouth, N.S.

Munda, I. 1977. The structure and distribution of *Gigartina stellata* (Stackh.) Batters and *Chondrus crispus* Stackh. associations in Icelandic waters. *Bot. Mar.* 20: 291-301.

Neish, I.C. and **Dunn, R.S.** 1971. *Atlantic Mariculture Ltd. rockweed report,* New Brunswick Dept. Fish. & Environ., Fredericton, N.B.

Niemeck, R.A. and **Mathieson, A.C.** 1978. Physiological studies of intertidal fucoid algae. *Bot. Mar.* 21: 221-227.

Novaczek, I. and **McLachlan, J.** 1986. Recolonization by algae of the sublittoral habitat of Halifax County, Nova Scotia, following the demise of sea urchins. *Bot. Mar.* 29: 69-73.

Novaczek, I., Bird, C.J. and **McLachlan, J.** 1987. Phenology and temperature tolerance of the red algae *Dasya baillouviana, Chondria baileyana, Griffithsia globifera,* and *Lomentaria baileyana,* in Nova Scotia. *Can. J. Bot.* 65: 57-62.

Owens, E.H. 1976. The effects of ice on the littoral zone at Richibucto Head, eastern New Brunswick. *Rev. Géogr. Montr.* 30: 95-104.

Owens, E.H. and **Bowen, A.J.** 1977. Coastal environments of the Maritime Provinces. *Mar. Sed.* 13: 1-31.

Platt, T. and **Irwin, B.** 1970. Primary productivity measurements in St. Margaret's Bay, 1968-1970. *Fish. Res. Board Can. Tech. Rept.* 203: 1-68.

Platt, T. and **Irwin, B.** 1972. Primary productivity and nutrients in St. Margaret's Bay, 1966. *Fish. Res. Board Can. Tech. Rept.* 327: 1-21.

Price, J.H. and **Farnham, W.F.** 1982. Seaweeds of the Faroes 3: Open shores. *Bull. Br. Mus. (Nat. Hist.) Bot.* 10: 153-225.

Pringle, J.D. and **Semple, R.E.** 1980. The benthic algal biomass, commercial harvesting and *Chondrus* growth and colonization off southwestern Nova Scotia. *Can. Tech. Rept. Fish. Aquat. Sci.* 954: 144-168.

Probyn, T.A. and **Chapman, A.R.O.** 1983. Summer growth of *Chordaria flagelliformis* (O.F. Mull.) C. Ag.: Physiological strategies in a nutrient-stressed environment. *J. Exp. Mar. Biol. Ecol.* 73: 243-271.

Prouse, N.J. 1983. Primary production measurements in the Bay of Fundy from March 1979 to November 1980. *Can. Tech. Rept. Fish. Aquat. Sci.* 1167: 1-82.

Roland, A.E. 1982. *Geological background and physiography of Nova Scotia.* N.S. Inst. Sci., Halifax, N.S.

Scheibling, R. 1986. Increased macroalgal abundance following mass mortalities of sea urchins *(Strongylocentrotus droebachiensis)* along the Atlantic coast of Nova Scotia. *Oecologia* (Berl.) 68: 186-198.

Schiel, D.R. and **Foster, M.S.** 1986. The structure of subtidal algal stands in temperate waters. *Ann. Rev. Oceanogr. Mar. Biol.* 24: 265-307.

South, G.R. 1984. A checklist of marine algae of eastern Canada. *Can. J. Bot.* 62: 680-704.

Southward, A.J. 1958. The zonation of plants and animals on rocky sea shores. *Biol. Rev.* 33: 137-177.

Southward, A.J. and **Southward, E.C.** 1978. Recolonization of rocky shores in Cornwall after use of toxic dispersants to clean up the Torrey Canyon spill. *J. Fish. Res. Board Can.* 35: 682-706.

Stasko, A.B., Amaratunga, T. and **Caddy, J.F.** 1977. Nineteen seventy-five Northumberland Strait project, Part II: Commercial shellfish data. *Fish. Mar. Serv. Manu. Rept.* 1432: 29.

Stasko, A.B., Campbell, A. and **Graham, D.E.** 1980. Sea urchin (*Strongylocentrotus droebachiensis*) distribution around western Nova Scotia. *Can. Tech. Rept. Fish. Aquat. Sci.* 954: 225-236.

Steele, D.H. 1983. Coarse sedimentary shores. In: *Marine and coastal systems of the Quoddy Region, New Brunswick.* (ed. M.L.H. Thomas.) *Can. Spec. Publ. Fish. Aquat. Sci.* 64: 74-94.

Stephenson, T.A. and **Stephenson, A.** 1954. Life between tide-marks in North America IIIB. Nova Scotia and Prince Edward Island: The geographical features of the region. *J. Ecol.* 42: 46-70.

Stephenson, T.A. and **Stephenson, A.** 1972. *Life between tidemarks on rocky shores.* W.H. Freeman, San Francisco.

Steven, D.M. 1974. Primary and secondary production in the Gulf of St. Lawrence. *Mar. Sci. Centre Manus. Rept. No. 26,* McGill University, Montreal, 1-116.

Sundene, O. 1962. The implications of transplant and culture experiments on the growth and distribution of *Alaria esculenta. Nytt. Mag. Bot.* 9: 155-174.

Sutcliffe, W.H. Jr., Loucks, R.H. and **Drinkwater, K.F.** 1976. Coastal circulation and physical oceanography of the Scotian Shelf and the Gulf of Maine. *J. Fish. Res. Board Can.* 33: 98-115.

Taylor, A.R.A. 1973. Studies of populations of *Chondrus crispus* (Irish moss) and *Furcellaria fastigiata* in Prince Edward Island waters during 1971. *Indust. Devel. Br. Environ. Can. Tech. Rept. Series* 65: 1-109.

Taylor, A.R.A. 1975. The *Chondrus crispus-Furcellaria fastigata* community at Campbell's Cove, Prince Edward Island. *Indust. Devel. Br., Fish. Mar. Serv., Environ. Can. Tech. Rept.* 88: 1-72.

Taylor, W.R. 1957. *Marine algae of the northeastern coast of North America.* University of Michigan Press, Ann Arbor, MI.

Thomas, M.L.H., Arnold, D.C. and **Taylor, A.R.A.** 1983. Rocky Intertidal Communities: In: *Marine and coastal systems of the Quoddy region, New Brunswick,* (ed., M.L.H. Thomas), *Can. Spec. Publ. Fish. Aquat. Sci.* 64: 35-73.

Underwood, A.J. 1978. A refutation of critical tide levels as determinants of the structure of intertidal communities on British shores. *J. Exp. Mar. Biol. Ecol.* 33: 261-276.

Underwood, A.J. and **Denley, E.J.** 1984. Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shores. In: *Ecological communities, conceptual issues and the evidence.* (eds. D.R. Strong Jr., D. Simberloff, L.G. Abele and A.B. Thistle), Princeton University Press, Princeton, NJ. 151-180.

Weiler, J.D.M. and **Keeley, J.R.** 1980. Monthly sea surface temperature for the Gulf of St. Lawrence. *Dept. Fish. Oceans, Mar. Environ. Data Service Tech. Rept.* 7: 1-43.

Wharton, W.G. 1980. *The extent of destructive grazing by the green sea urchin Strongylocentrotus droebachiensis on the eastern and southwestern shores of Nova Scotia.* Fish. and Oceans Canada. DSS Contract No. ISC 7900058, Halifax, N.S.

Wharton, W.G. and **Mann, K.H.** 1981. Relationship between destructive grazing by the sea urchin *Strongylocentrotus droebachiensis* and the abundance of American lobster *Homarus americanus* on the Atlantic coast of Nova Scotia. *Can. J. Fish. Aquat. Sci.* 38: 1339-1349.

Widdowson, T.B. 1965. A survey of the distribution of intertidal algae along a coast transitional in respect to salinity and tidal factors. *J. Fish. Res. Board Can.* 22: 1425-1454.

Wilce, R.T. 1959. The marine algae of the Labrador peninsula and northwest Newfoundland (ecology and distribution). *Nat. Mus. Can. Bull.* 158, *Biol. Ser.* 56, 1-103.

Wilson, J.S., Bird, C.J., McLachlan, J. and **Taylor, A.R.A.** 1979. An annotated checklist and distribution of benthic marine algae of the Bay of Fundy. *Memorial Univ. Nfld. Occ. Pap. Biol.* No. 2: 1-65.

Woelkerling, W.J. 1973. Morphology and systematics of the *Audouinella* complex (Acrochaetiales, Rhodophyta) in northeastern United States. *Rhodora* 75: 1-621.

Yabu, H. 1978. Chromosome numbers in species of *Porphyra* from Nova Scotia, Canada. *Jap. J. Phycol.* 26: 97-104.

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Appendix 1

List of surveyed sites, indicating the type of transect (I = intertidal, S = sublittoral), dates of surveys and the wave exposure (Table II), geology, presence of sea urchins (o = none seen, 1 = few, 2 = common or abundant), spring-tide range and intertidal slope in each locality, nd = no data.

Site No. & Name	Transect type: da-mo-yr	Wave Exp Index	Geology	Sea Urch	Tide Range (m)	Slope (m m^{-1})
BAY OF FUNDY: shores 1-2, 5-7						
1 Scots Bay	I, 13-05-77	2	basalt	0	14.6	0.09
2 Cape D'Or	I, 02-07-77	3	basalt	0	13.0	0.19
3 Hampton	I, 14-06-84	3	basalt	0	11.6	0.08
	S, 22-08-84			2		
4 Martin Head	I, 06-07-78	3	sandstone	0	11.1	0.16
5 Quaco Head	I, 05-07-78	2	sandstone	0	11.0	0.08
6 Delap Cove	I, 31-05-77	3	basalt	0	9.8	0.2
7 Cape Spencer	I, 04-07-78	4	sandstone	0	.9	0.2
	S, 05-07-78			0		
8 Musquash Head	I, 24-05-78	4	basalt	0	9.1	0.15
9 Welch Cove	I, 23-05-78	3	basalt	0	8.2	0.01
10 Deer Point	I, 20-06-78	1	basalt	2	8.1	0.23
11 E. Quoddy Head	I, 20-06-78	3	basalt	0	7.7	0.21
	S, 18-07-78			2		
11b	S, 18-07-78			0		
12 Dinner Head	I, 21-06-78	3	basalt	0	7.7	0.06
	S, 18-07-78			0		
13 Mascabin Point	I, 23-05-78	2	basalt	2	.8	0.2
	S, 23-05-78			2		
14 Gulliver Cove	I, 03-07-77	2	basalt	0	7.9	0.07
15 Sandy Cove	I, 02-06-77	3	basalt	0	7.8	0.09
	S, 02-06-77			0		
16 Ingalls Head	I, 22-06-78	3	basalt	0	7.3	0.04
	S, 22-06-78			0		
17 Swallowtail Light	I, 23-06-78	3	shale	0	7.3	0.29
	S, 23-06-78			0		
18 Dark Harbour	I, 22-06-78	4	basalt	0	6.0	0.1
	S, 20-07-78			0		

Appendix 1 (Cont'd.)

Site No. & Name	Transect type: da-mo-yr	Wave Exp Index	Geology	Sea Urch	Tide Range (m)	Slope ($m\ m^{-1}$)
19 South Head Beach	I, 21-06-78	4	basalt	0	6.0	0.08
	S, 26-06-78			2		
20 North Point	I, 04-06-77	3	basalt	0	6.6	0.12
ATLANTIC:						
Funday approaches: shores 8-9						
21 Meteghan	I, 02-06-77	3	slate	0	6.2	0.08
	S, 02-06-77			0		
22 Cape St. Mary	I, 01-08-77	3	slate	0	5.8	0.04
23 Burns Point	I, 03-08-77	3	slate	0	5.7	0.05
24 Cheggogin Point	I, 02-08-77	3	slate	0	5.7	0.04
25 Chebogue Point	I, 02-08-77	4	slate	0	4.7	0.02
	S, 02-08-77			0		
26 Comeau Hill	I, 03-08-77	2	granite	0	4.7	0.02
27 Lower E. Pubnico	I, 17-05-84	2	granite	0	4.5	0.1
28 St. Ann Point	I, 16-05-84	2	slate	0	4.2	0.05
29 West Head	I, 17-05-84	2	granite	0	3.8	0.14
Eastern Atlantic: shores 10, 11-15						
30 Ingomar Point	I, 18-05-84	2	granite	0	2.7	0.05
	S, 31-07-84			0		
31 Western Head	I, 10-07-84	4	quartzite	0	2.4	0.07
	S, 10-07-84			0		
32 Ovens	I, 05-07-84	3	slate	0	2.2	0.02
	S, 05-07-84			0		
33 Fox Point	I, 19-06-84	2	granite	0	2.2	0.08
	S, 16-08-84			0		
34 Northwest Cove	I, 08-06-84	1	granite	0	2.1	0.22
	S, 16-08-84	1		0		
34b	S, 16-08-84	2		0		

Appendix 1 (Cont'd.)

Site No. & Name	Transect type: da-mo-yr	Wave Exp Index	Geology	Sea Urch	Tide Range (m)	Slope ($m\ m^{-1}$)
35 Prospect	I, 07-06-84	4	granite	0	2.1	0.13
	S, 13-08-84			0		
36 Portuguese Cove	I, 06-06-84	3	granite	0	2.1	0.1
37 Boutiliers Island	I, 12-07-74	1	slate	0	2.0	0.05
	S, 12-07-84			0		
38 Sober Island (W)	I, 11-07-84	3	slate	0	2.0	0.05
	S, 11-07-84			0		
39 Tor Bay	I, 17-07-84	4	quartzite	0	1.95	0.08
	S, 19-07-84			1		
40 Rocky Bay	I, 19-07-74	3	sandstone	0	2.0	0.04
	S, 19-07-84			2		
41 Gooseberry Cove	S, 18-07-84	4	shale	2	1.7	nd
42 Neal Cove	I, 18-07-84	2	sandstone	0	1.7	0.04
	S, 18-07-84			1		
43 Point Aconi	S, 08-08-84	3	sandstone	2	1.25	nd
44 Wreck Point	S, 08-08-84	3	granite	1	1.3	nd
SOUTHERN GULF OF ST LAWRENCE: shores 17, 20-22						
45:East Point T2	S, 16-05-77	3	sandstone	2	1.1	nd
46:T3	S, 16-05-77	3	sandstone	2	1.1	nd
47:T4	S, 16-05-77	3	sandstone	2	1.1	nd
48:T6	S, 13-05-77	3	sandstone	2	1.1	nd
49:T7	S, 13-05-77	3	sandstone	2	1.1	nd
50:T8	S, 13-05-77	3	sandstone	1	1.1	nd
51:T10	S, 09-05-77	3	sandstone	2	1.1	nd
52:T11	S, 17-05-77	3	sandstone	2	1.1	nd
53:T12	S, 17-05-77	3	sandstone	1	1.1	nd
54:T14	S, 19-05-77	3	sandstone	1	1.1	nd
55:T18	S, 20-05-77	3	sandstone	1	1.1	nd
56:T19	S, 20-05-77	3	sandstone	1	1.1	nd
57:T21	S, 20-05-77	3	sandstone	1	1.1	nd

Appendix 1 (Cont'd.)

Site No. & Name	Transect type: da-mo-yr	Wave Exp Index	Geology	Sea Urch	Tide Range (m)	Slope ($m\ m^{-1}$)
58:T24	S, 23-05-77	3	sandstone	1	1.1	nd
59:T25	S, 23-05-77	3	sandstone	1	1.1	nd
60:T26	S, 25-05-77	3	sandstone	2	1.1	nd
61:T29	S, 24-05-77	3	sandstone	1	1.1	nd
62:T30	S, 24-05-77	3	sandstone	1	1.1	nd
63:T32	S, 24-05-77	3	sandstone	1	1.1	nd
64:T33	S, 25-05-77	3	sandstone	2	1.1	nd
65:T34	S, 31-05-77	3	sandstone	1	1.1	nd
66:T35	S, 31-05-77	3	sandstone	2	1.1	nd
67:T36	S, 31-05-77	3	sandstone	2	1.1	nd
68:T37	S, 31-05-77	3	sandstone	1	1.1	nd
69:T38	S, 01-06-77	3	sandstone	2	1.1	nd
70:T39	S, 31-05-77	3	sandstone	1	1.1	nd
71:T40	S, 01-06-77	3	sandstone	2	1.1	nd
72:T41	S, 01-06-77	3	sandstone	1	1.1	nd
73:T42	S, 01-06-77	3	sandstone	1	1.1	nd
74:T44	S, 02-06-77	3	sandstone	1	1.1	nd
75:T45	S, 08-06-77	3	sandstone	1	1.1	nd
76:T46	S, 08-06-77	3	sandstone	1	1.1	nd
77:T48	S, 07-06-77	3	sandstone	1	1.1	nd
78:T49	S, 09-06-77	3	sandstone	2	1.1	nd
79:T51	S, 09-06-77	3	sandstone	1	1.1	nd
80 St. Peters Bay:T57	S, 14-06-77	3	sandstone	2	1.1	nd
81 Doyle's Cove	S, 03-08-85	3	sandstone	1	1.1	nd
82 Cape Tryon	S, 02-07-85	3	sandstone	1	1.1	nd
83 Anglo	S, 29-07-85	3	sandstone	1	1.2	nd
84 Seacow Pond	S, 16-07-85	3	sandstone	1	1.2	nd
85 Norway	S, 31-07-85	3	sandstone	1	1.2	nd
86 Miminegash	S, 19-08-85	3	sandstone	1	1.2	nd
87 Cape Wolfe	S, 15-08-85	3	sandstone	0	1.3	nd
88 Cape Egmont	S, 17-06-85	2	sandstone	0	1.4	nd
89 Amherst Point	S, 14-08-85	2	sandstone	0	2.4	nd

Appendix 2

List of species in each sector of 3 coastal environments of the Maritime Provinces, Canada, indicating the vertical distribution of each species. For locations of shores and sites see Fig 2. P = present, with height or depth undocumented; 1 = rockpool above MWL; 2 = rockpool below MWL; A = above HHWN; B = HHWN to LLWN; C = LLWN to CD; D = CD to 2 m depth; E = below 2 m depth; * = wave exposure 4 only (see Table V); + = site 39 and shore 12 only. Data in brackets are from one of the following sources: Adey (1964, 1965), Cardinal (1968), Edelstein *et al.* (1969), Wilson *et al.* (1979), Novaczek and McLachlan (1986), Lobban and Hanic (1984), NRCC herbarium, C.J. Bird unpublished records. Nomenclature follows South (1984) except where noted (see footnotes).

Sector:	Inner Fundy	Outer Fundy	Fundy Approaches	Eastern Atlantic	Open Gulf	Lower Gulf
Shores:	1-5	6-7	8-9	10-15	16, 17, 20	18, 19, 21, 22
Sites:	1-6	7-20	21-29	30-44	45-83	84-89
Phaeophyta						
Acrothrix novae-angliae	D	-	(P)	(DE)	E	(D)E
Agarum cribrosum	CD	2CDE	(E)	E	E	E
Alaria esculenta	CD	2CDE	D	1*2B*CDE	-	-
Ascocyclus distromaticus	-	-	-	-	D	(E)
Ascophyllum nodosum	ABC	12ABC	1ABCD	2ABC	(P)	(P)
Chorda filum	(P)	(P)	ED	CDE	DE	(CD)E
Chorda tomentosa	C	2CDE	CD	CDE	E	(P)
Chordaria flagelliformis	2C	12BCD	12CD(E)	12BCDE	(B)CDE	E
Delamarea attenuata	-	-	-	(P)	DE	(P)
Desmarestia aculeata	(P)	CDE	(DE)	C*DE	E	E
Desmarestia viridis	2CD	CDE	2CDE	2CDE	E	E
Desmotrichum undulatum	-	P	(P)	(P)	DE	(D)
Dictyosiphon eckmanii	(P)	(P)	(P)	(1)	D	-
Dictyosiphon foeniculaceous	2	12B	2D	12BCDE	CDE	(D)E
Ectocarpus confervoides	-	(P)	(P)	E	(P)	(P)
Ectocarpus fasciculatus	(P)	(P)	(E)	CD	DE	-
Ectocarpus siliculosus	C	C	12D(E)	1*2B*CDE	DE	(D)E
Elachista fucicola	BC	BC	BCD	2 BCD	DE	-
Elachista lubrica	-	P	2	(P)	-	-
Eudesme virescens	-	(P)	2(D)	DE	(P)	-
Feldmannia irregularis	-	-	-	-	E	(P)
Fucus distichus	1	1	1	1	-	(P)

Appendix 2 (Cont'd.)

Sector:	Inner Fundy	Outer Fundy	Fundy Approaches	Eastern Atlantic	Open Gulf	Lower Gulf
Shores:	1-5	6-7	8-9	10-15	16, 17, 20	18, 19, 21, 22
Sites:	1-6	7-20	21-29	30-44	45-83	84-89
Fucus evanescens	BC	BC	2CD	BCDE	DE	(D)
Fucus serratus	-	-	BCD	CDE	DE	(BC)D
Fucus spiralis	1A	A	1AB	AB	(P)	(B)
Fucus vesiculosus	ABC	1ABC	ABC	2ABCDE	(B)CDE	(B)DE
Giffordia granulosa	-	-	-	(P)	E	(E)
Giffordia ovata	-	-	-	-	E	(E)
Giffordia sandriana	-	-	-	-	E	-
Giffordia sp.	-	-	-	BCD	E	-
Halopteris scoparia	-	-	(P)	(E)	DE	DE
Haplospora globosa	-	(P)	-	2(E)	DE	-
Isthmoplea sphaerophora	(P)	BC	B	B*CD	-	(P)
Laminaria digitata	2C	12CDE	2CDE	1*2B*CDE	E	-
Laminaria saccharina[1]	2C	2CDE	2CDE	1*2CDE	E	E
Laminariocolax tomentosoides	-	(P)	C	(P)	-	-
Leathesia difformis	(P)	1D	2CDE	12BC	D	(P)
Leptonematella fasciculata	B	P	BC	2BC	E	(P)
Litosiphon pusillus	-	-	-	-	DE	(P)
Melanosiphon intestinalis	B	P	(P)	-	-	-
Microspongium globosum	-	P	(P)	(P)	-	(P)
Myriocladia lovenii	-	-	-	(P)	DE	(E)
Myrionema strangulans	-	P	(P)	E	D	-
Myriotrichia filiformis	-	(P)	-	(P)	D	(P)
Petalonia fascia	12C	2CD	1CD	12B*C	(B)DE	D(E)
Petalonia zosterifolia	-	(P)	BC	(P)	CDE	(D)
Pilayella littoralis	B	2BC	1BC	2A*BCD	(D)E	(E)
Pseudolithoderma sp.	-	A	B	(P)	-	-
Punctaria latifolia	-	P	P	1DE	(B)DE	(E)
Punctaria plantaginea	-	12	C	1*D	DE	(D)
Ralfsia clavata	-	P	P	CD	DE	(D)E
Ralfsia fungiformis	BC	2C	2	2	-	-

Appendix 2 (Cont'd.)

Sector:	Inner Fundy	Outer Fundy	Fundy Approaches	Eastern Atlantic	Open Gulf	Lower Gulf
Shores:	1-5	6-7	8-9	10-15	16, 17, 20	18, 19, 21, 22
Sites:	1-6	7-20	21-29	30-44	45-83	84-89
Ralfsia verrucosa	(1)	2	2B	B	(B)CE	(P)
Saccorhiza dermatodea	(P)	2CDE	DE	1*2CDE	E	E
Scytosiphon dotyi	2	C	-	-	-	-
Scytosiphon lomentaria	1C	12BC	12CD	12BCDE	(B)CDE	(12BE)D
Sorocarpus micromorus	-	-	-	DE	DE	-
Sphacelaria cirrosa	-	(P)	(P)	BCD	D	(D)E
Sphacelaria fusca	-	(P)	-	2	-	-
Sphacelaria plumosa	-	P	(P)	E	DE	E
Sphacelaria radicans	-	C	E	-	DE	DE
Sphacelaria rigidula	(P)	P	(P)	1	-	(E)
Sphaerotrichia divaricata	(P)	(P)	CD	B*	DE	D(E)
Spongonema tomentosum	(P)	(P)	B	B*C	(P)	-
Stictyosiphon griffithsianus	-	-	-	(P)	E	(P)
Stictyosiphon tortilis	-	-	-	-	DE	(D)E
Striaria attenuata	-	-	-	-	E	(E)
Tilopteris mertensii	-	-	-	(E)	E	(E)
Chlorophyta						
Blidingia chadefaudii	-	P	-	-	-	-
Blidingia marginata	P	(P)	B	(P)	-	(P)
Blidingia minima	(A)	1	A	1AB	(P)	(A)
Chaetomorpha aerea	-	-	-	(P)	CD	(D)
Chaetomorpha brachygona[2]	(P)	-	-	(1)	DE	DE
Chaetomorpha cannabina	2	1BC	C	(1)	-	(P)
Chaetomorpha linum	-	(P)	D(E)	BE	(P)	(P)
Chaetomorpha melagonium	12CD	12CDE	CDE	1*DE	DE	E
Chaetomorpha picquotiana	-	P	E	(P)	-	E
Cladophora albida	D	(P)	C	2CE	DE	E
Cladophora crystallina[5]	(P)	1	2	(P)	-	-
Cladophora rupestris	C	B	2BC	1*BC	-	-

Appendix 2 (Cont'd.)

Sector:	Inner Fundy	Outer Fundy	Fundy Approaches	Eastern Atlantic	Open Gulf	Lower Gulf
Shores:	1-5	6-7	8-9	10-15	16, 17, 20	18, 19, 21, 22
Sites:	1-6	7-20	21-29	30-44	45-83	84-89
Cladophora sericea	1	(P)	2(D)	12BCDE	DE	(B)DE
Derbesia vaucheriaformis	-	-	-	DE	-	-
Enteromorpha compressa	1	P	-	(P)	-	(P)
Enteromorpha flexuosa ssp. paradoxa	P	P	-	C	(P)	(P)
Enteromorha groenlandica	(P)	B	-	(P)	-	-
Enteromorpha intestinalis	1	12	1	1B	C	(ABD)E
Enteromorpha linza	1	12BC	B	(P)	(B)	(P)
Enteromorpha prolifera ssp. prolifera	-	P	(P)	(P)	(P)	(P)
Monostroma grevillii	12C	12CD	(P)	E	(P)	(P)
Monostroma pulchrum	(P)	12C	(P)	B*CD	-	-
Prasiola crispa	A	-	-	(P)	-	-
Prasiola stipitata	A	A	(P)	A	-	(P)
Pringsheimiella scutata	-	(P)	-	(P)	P	(DE)
Pseudendoclonium submarinum	(P)	-	-	(P)	DE	(D)
Rhizoclonium riparium[2]	P	2	2C	1	D	(P)
Rhizoclonium tortuosum[2]	1	-	-	2A*DE	E	(D)E
Spongomorpha aeruginosa	-	P	1B	BCDE	DE	(E)
Spongomorpha arcta	2	12BCD	BCD	1*CDE	E	D(E)
Spongomorpha sonderi	C	P	(P)	-	-	-
Spongomorpha spinescens	BC	12BCD	(P)	12BCDE	-	-
Ulothrix flacca	A	1A	(P)	(P)	(B)C	-
Ulothrix laetivirens	A	(P)	-	A	-	-
Ulothrix speciosa	(P)	AB	-	(P)	-	-
Ulothrix subflaccida	(P)	-	AB	A	-	(P)
Ulva lactuca	(12C)	12CD	(P)	12A*BDE	(B)DE	(D)E
Ulva rigida	2	2D	C(D)	(P)	D	-
Ulvaria obscura v. blyttii	1	2CD	E	(P)	E	(E)
Ulvaria oxysperma	(P)	C	2BC	1*2B*CE	(P)	-
Urococcus foslieanus	-	-	-	A	-	-
Urospora penicilliformis	A	B	AB	(P)	(P)	-

Appendix 2 (Cont'd.)

Sector:	Inner Fundy	Outer Fundy	Fundy Approaches	Eastern Atlantic	Open Gulf	Lower Gulf
Shores:	1-5	6-7	8-9	10-15	16, 17, 20	18, 19, 21, 22
Sites:	1-6	7-20	21-29	30-44	45-83	84-89
Urospora wormskjoldii	B	ABC	(C)	(P)	(B)	-
Vaucheria sp.	-	-	E	-	-	(E)
Rhodophyta						
Ahnfeltia plicata	2C	12CD	2BC(DE)	1*2CDE	DE	DE
Antithamnion cruciatum	(P)	DE	(P)	E	DE	DE
Antithamnion plumula	-	-	(P)	E	-	-
Antithamnionella floccosa	(P)	C	(P)	B*CDE	(P)	-
Audouinella dasyae	-	-	-	-	E	-
Audouinella daviesii	-	(P)	(P)	1B	DE	(D)E
Audouinella microscopica[3]	-	-	(P)	1D	-	E
Audouinella purpurea	(P)	BC	(P)	(P)	-	-
Audouinella saviana	-	(P)	B	-	E	E
Audouinella secundata	B	P	(P)	(P)	C	(P)
Audouinella spetsbergensis	-	-	-	-	E	E
Bangia atropurpurea	(P)	P	B	1AB	(B)	-
Bonnemaisonia hamifera[7]	D	-	D	2BCDE	DE	DE
Callithamnion corymbosum	-	-	(P)	E	E	(D)E
Callithamnion hookeri	-	-	-	E	-	-
Callithamnion tetragonum	-	-	-	(P)	E	(P)
Callophyllis cristata	C	CDE	(DE)	E	DE	E
Ceramium deslongchampii v. hooperi	C	C	(E)	E	-	-
Ceramium elegans	(P)	(P)	(P)	-	(P)	E
Ceramium rubrum	D	12BCDE	2CDE	2CDE	DE	DE
Ceratocolax hartzii	-	(P)	-	(E)	E	(E)
Chondria baileyana	-	-	-	-	E	(DE)
Chondrus crispus	12BC	12BCDE	12BCDE	12BCDE	CDE	(12BC)DE
Choreocolax polysiphoniae	P	P	(P)	BC	-	E
Clathromorphum circumscriptum	(P)	(P)	C	C	E	-
Clathromorphum compactum	DE	(P)	(P)	2DE	E	-

Appendix 2 (Cont'd.)

Sector:	Inner Fundy	Outer Fundy	Fundy Approaches	Eastern Atlantic	Open Gulf	Lower Gulf
Shores:	1-5	6-7	8-9	10-15	16, 17, 20	18, 19, 21, 22
Sites:	1-6	7-20	21-29	30-44	45-83	84-89
Corallina officinalis	12CD	12BCDE	12BCDE	2BCDE	DE	(BC)DE
Cystoclonium purpureum	1BC	1CD	2CDE	2B*CDE	DE	E
Dermatolithon pustulatum	(P)	C	CD	-	E	D
Devaleraea ramentacea	12	12BCD	BCD	1*2B*CDE	(CD)	-
Dilsea integra[4]	-	-	-	E	E	E
Dumontia contorta	12BC	12C	12CD	1*2BCDE	E	(P)
Erythrotrichia carnea	-	(P)	P	2	(P)	(P)
Fimbrifolium dichotomum	-	CDE	-	E	E	-
Fosliella farinosa (Lamour.) M. Howe[12]	-	P	P	(P)	-	(P)
Furcellaria lumbricalis	-	-	-	DE[+]	DE	(D)E
Gloiosiphonia capillaris	(P)	-	(DE)	DE	-	-
Harveyella mirabilis	(P)	P	P	E	DE	E
Hildenbrandia spp.[8]	12ABC	12BCDE	12ABC	12ABCDE	E	-
Leptophytum laeve	-	E	(P)	-	E	E
Lithophyllum orbiculatum	E	(P)	(P)	E	-	-
Lithothamnion glaciale	E	(P)	E	DE	E	E
Lithothamnion lemoineae	-	(P)	(P)	C*DE	E	-
Mastocarpus stellatus[13]	2BCD	2BCDE	CD	BC	-	-
Membranoptera alata	C	2CDE	(E)	E	E	E
Odonthalia dentata	(P)	P	(E)	E	E	E
Palmaria palmata	BC	2BCDE	BCDE	1*2A*BCDE	DE	E
Petrocelis cruenta[11]	BC	BC	C	(P)	E	-
Peyssonnelia rosenvingii	E	P	(P)	E	E	-
Phycodrys rubens	2CDE	2BCDE	2CDE	E	DE	E
Phyllophora pseudoceranoides	CDE	12CDE	2CDE	E	E	E
Phyllophora truncata	(P)	2DE	C(DE)	CE	DE	DE
Phymatolithon laevigatum	(P)	(P)	BCD	DE	E	-
Phymatolithon lenormandii	(P)	(P)	B	C	-	-
Phymatolithon rugulosum	(P)	(P)	B	DE	E	-

Appendix 2 (Cont'd.)

Sector:	Inner Fundy	Outer Fundy	Fundy Approaches	Eastern Atlantic	Open Gulf	Lower Gulf
Shores:	1-5	6-7	8-9	10-15	16, 17, 20	18, 19, 21, 22
Sites:	1-6	7-20	21-29	30-44	45-83	84-89
Plumaria elegans	C	12BC	P	(P)	DE	E
Pneophyllum lejolisii	-	(P)	-	(P)	E	(D)
Polyides rotundus	2C	12CDE	BCDE	DE	DE	DE
Polysiphonia elongata	-	-	-	E	(P)	(P)
Polysiphonia fibrillosa	-	-	(E)	BDE	(P)	(P)
Polysiphonia flexicaulus	-	P	(P)	CDE	DE	DE
Polysiphonia harveyi	(P)	(P)	E	CDE	DE	(D)E
Polysiphonia lanosa	BC	ABC	BC	ABC	-	-
Polysiphonia nigrescens	D	DE	E	BCDE	DE	DE
Polysiphonia novaeangliae[5]	-	DE	D	2CDE	D	DE
Polysiphonia subtilissima	-	-	-	1E	-	(P)
Polysiphonia urceolata	1CD	CDE	DE	1*2CDE	DE	(D)E
Porphyra leucosticta[9]	(P)	C	-	(P)	-	-
Porphyra linearis	(P)	(P)	-	(P)	C	-
Porphyra miniata[10]	1AB	2CD	C(D)E	BDE	-	-
Porphyra umbilicalis[9]	AB	2ABC	A	CE	(P)	(P)
Ptilota serrata	(P)	CDE	(E)	E	E	E
Rhodomela confervoides[6]	C	2CDE	2DE	BCDE	DE	DE
Rhodophysema elegans	-	(P)	-	E	E	E
Scagelia corallina	(P)	DE	-	E	E	DE
Scinaia forcellata	-	-	P	-	(P)	-
Stylonema alsidii	-	-	-	(P)	DE	DE
Fauna						
Balanus, Pseudobalanus spp.	AB	1ABC	12B	ABC	(AB)	(BC)E

Appendix 2 (Cont'd.)

Sector:	Inner Fundy	Outer Fundy	Fundy Approaches	Eastern Atlantic	Open Gulf	Lower Gulf
Shores:	1-5	6-7	8-9	10-15	16, 17, 20	18, 19, 21, 22
Sites:	1-6	7-20	21-29	30-44	45-83	84-89
Mytilus edulis	BC	BE	2BD	12BCDE	(CD)E	(BD)E
Strongylocentrotus droebachiensis	DE	CDE	-	DE	E	E

[1] Including *L. longicruris.*
[2] Sensu Blair (1983).
[3] Sensu Woelkerling (1973).
[4] Lindstrom (1985).
[5] Sensu Taylor (1957).
[6] Including *R. lycopodioides.*
[7] Tetrasporophyte only, except for occurrence of gametophytes in Fundy approaches and eastern Atlantic.
[8] *H. crouanii* and/or *H. rubra* (C. Maggs pers. comm.).
[9] Sensu Yabu (1978). Validity of nomenclature is in question (Mitman pers. comm).
[10] Validity of nomenclature is in question (Chen pers. comm.).
[11] Guiry et al. (1984).
[12] Chamberlain (1983).
[13] See also tetrasporophytic phase, *Petrocelis cruenta.*

Appendix 3

Vertical distributions of macroalgae at Fundy sites 2-19; Fundy Approaches (Atlantic) sites 21-26, eastern Atlantic sites 30-44; and Gulf sites 65-89. Range of dominance or co-dominance (species code in large letters) indicated by a bar; presence beyond this range, by a line. Species common within the same range encoded in small letters. Rock pool and epiphytic species excluded. Intertidal heights relative to tidal levels, sublittoral excluded. Intertidal heights relative to tidal levels, sublittoral depths indicate m below CD. Species are encoded as follows:

BROWN ALGAE

Ac *Agarum cribrosum*
Ae *Alaria esculenta*
An *Ascophyllum nodosum*
Cf *Chorda filum*
Ch *Chordaria flagelliformis*
Da *Desmarestia aculeata*
Df *Dictyosiphon foeniculaceous*
Dv *Desmarestia viridis*
Fe *Fucus evanescens*
Fr *F. serratus*
Fs *F. spiralis*
Fv *F. vesiculosus*
Hs *Halopteris scoparia*
Ld *Laminaria digitata*
Ls *L. saccharina/longicruris*
Sd *Saccorhiza dermatodea*
Sl *Scytosiphon lomentaria*

GREEN ALGAE

Bm *Blidingia minima*
Cm *Chaetomorpha melagonium*
Sa *Spongomorpha arcta*
Ss *S. spinescens*
Uf *Ulothrix flacca*
Ul *Ulva lactuca*
Ur *Urospora spp.*

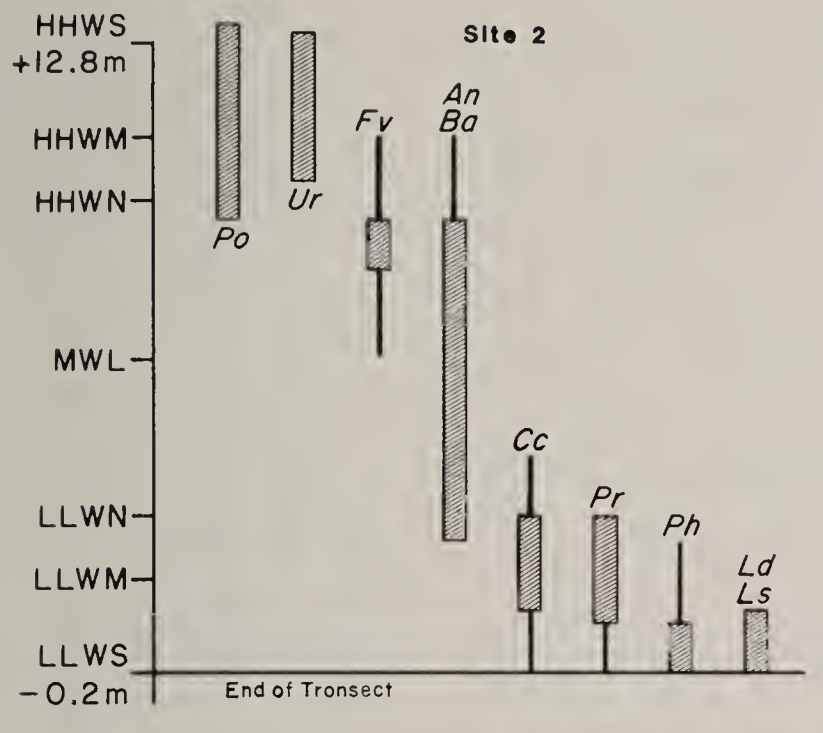
RED ALGAE

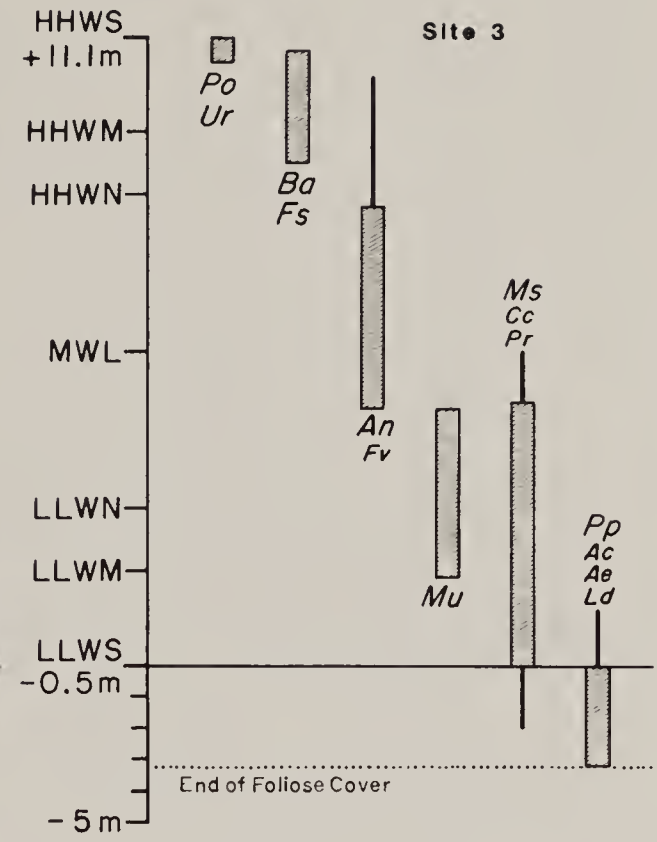
Ap *Ahnfeltia plicata*
Cc *Chondrus crispus*
Co *Corallina officinalis*
Cy *Cystoclonium purpureum*
Di *Dilsea integra*
Dr *Devaleraea ramentacea*
Fl *Furcellaria lumbricalis*
Ms *Mastocarpus stellatus*
Od *Odonthalia dentata*
Pa *Palmaria palmata*
Pb *Polysiphonia subtilissima*
Pe *P. elongata*
Ph *Phycodrys rubens*
Pn *Polysiphonia nigrescens*
Po *Porphyra spp.*
Pp *Phyllophora pseudoceranoides*
Pr *Polyides rotundus*
Ps *Ptilota serrata*
Pt *Phyllophora truncata*
Pu *Polysiphonia urceolata*
Rc *Rhodomela confervoides*
Sc *Scagelia corallina*

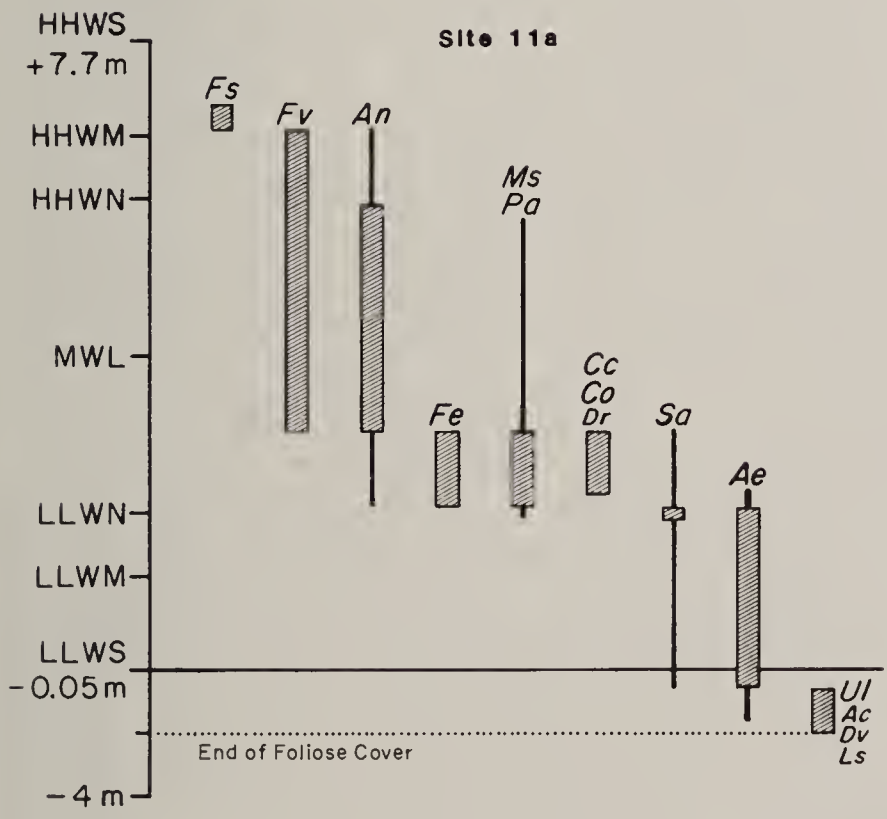
ANIMALS

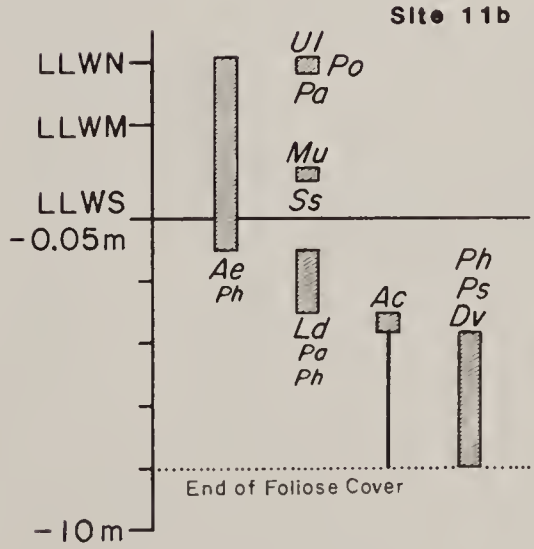
Ba *Barnacles*
Mu *Mussels*

Appendix 3 (Cont'd.)









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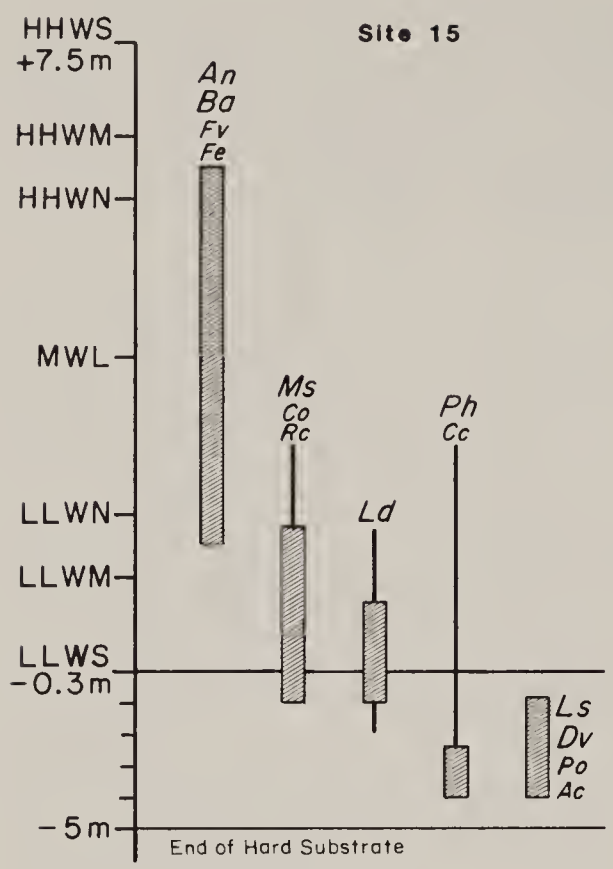

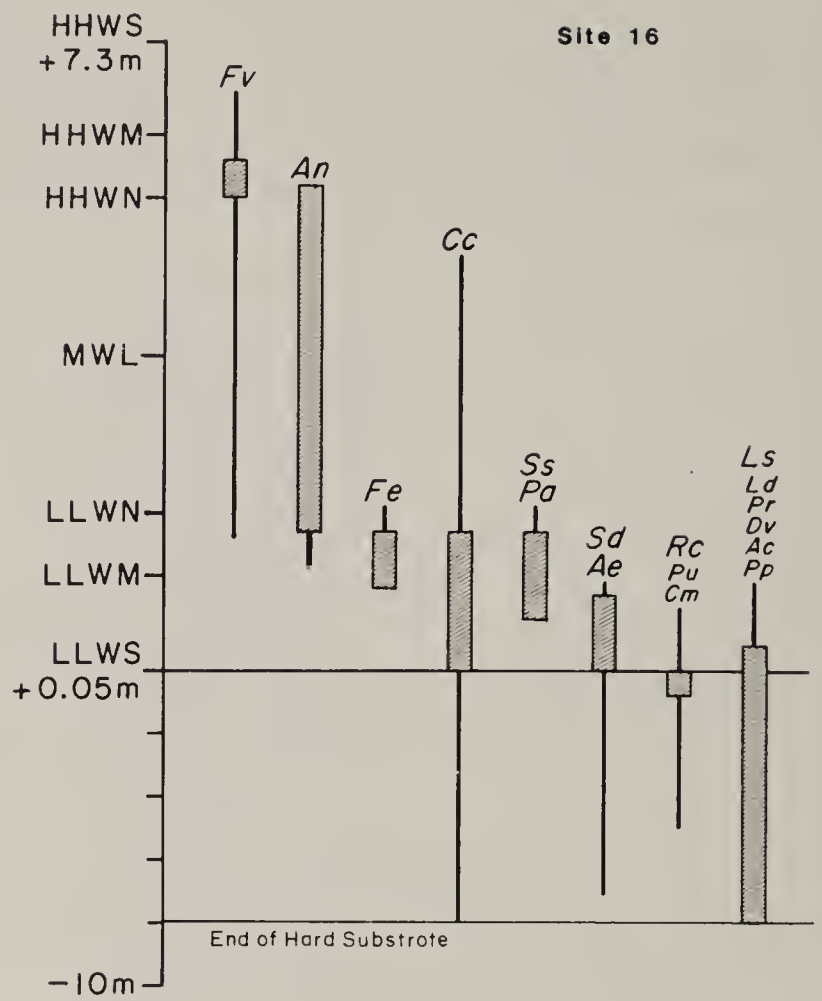

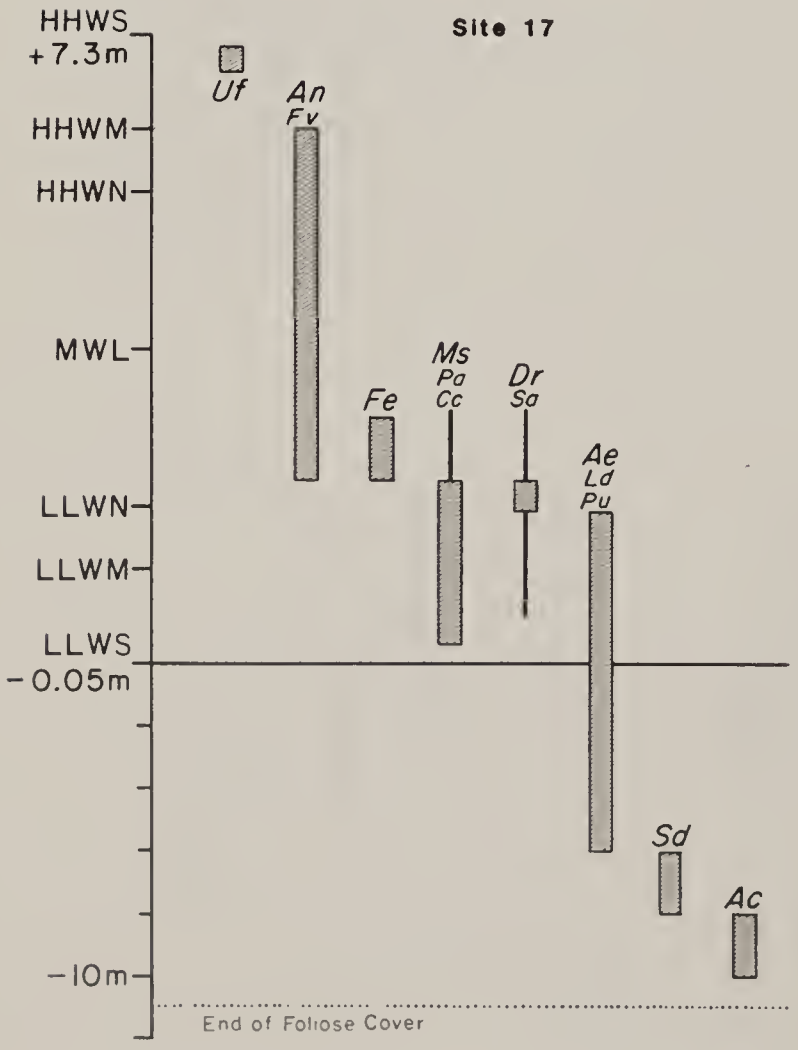

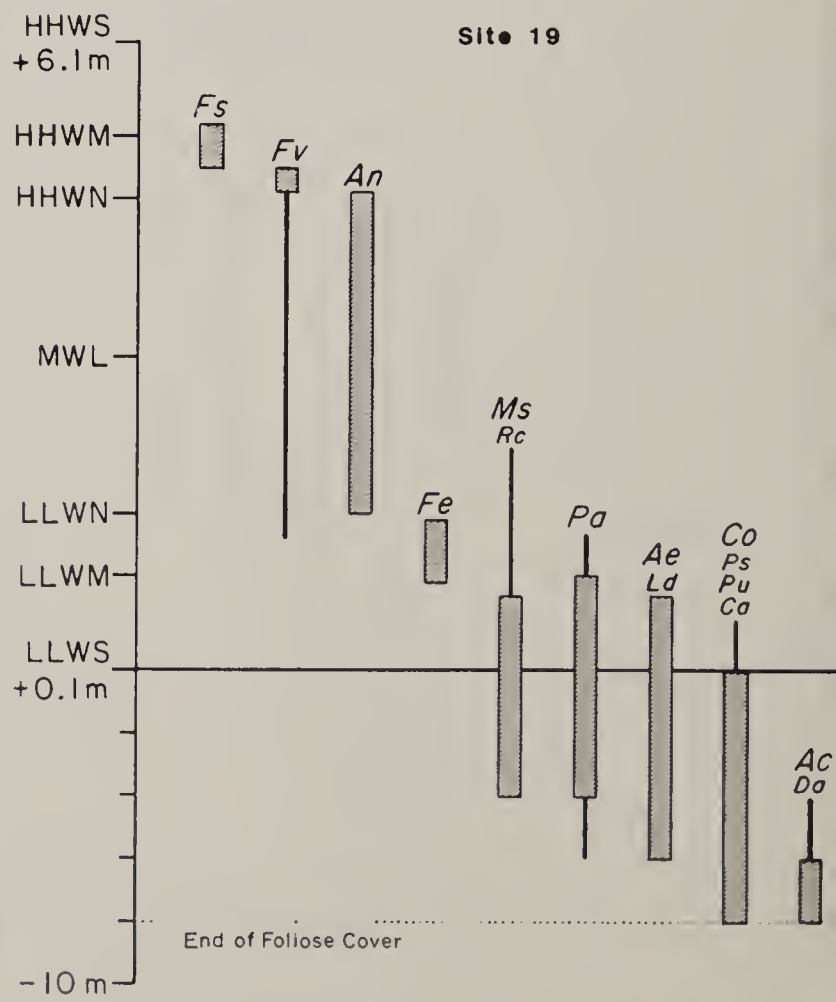

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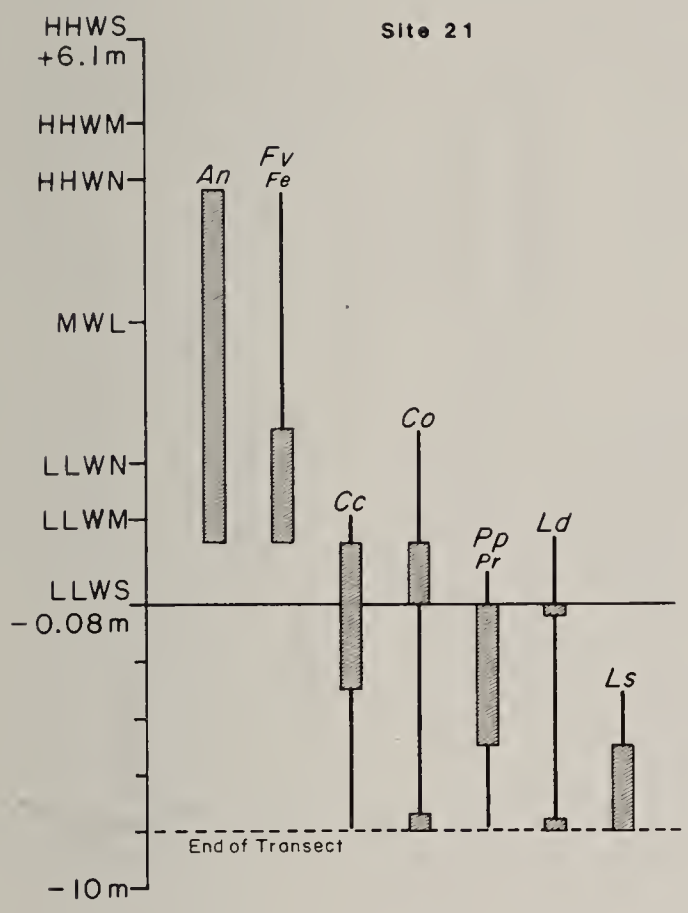

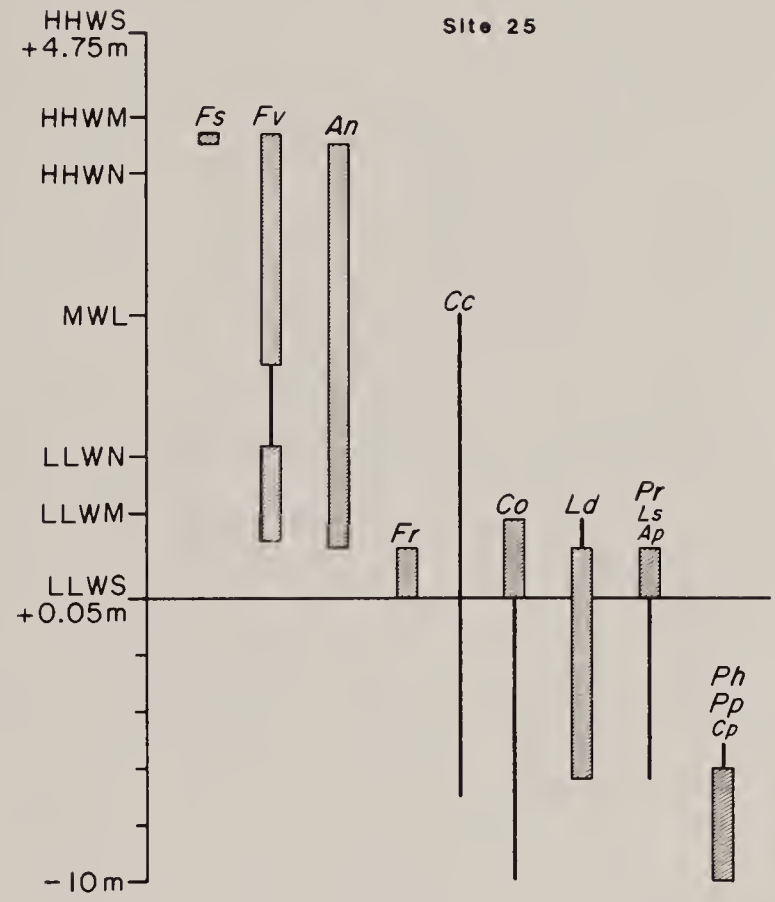

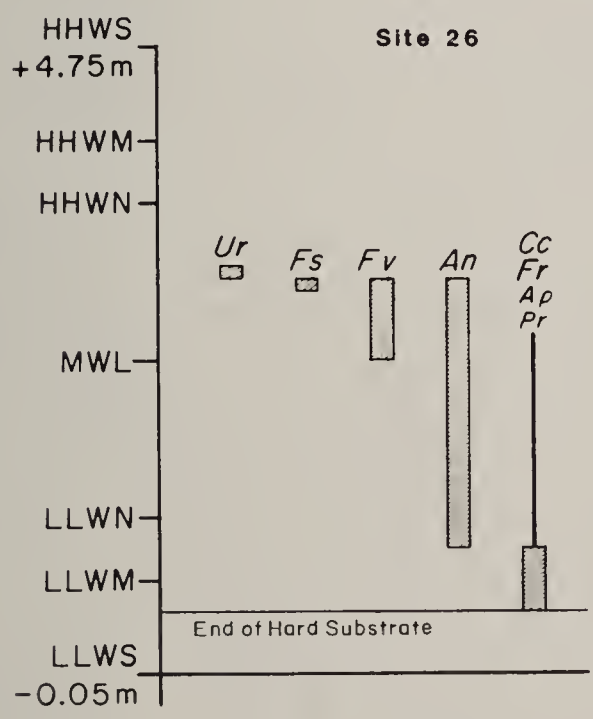

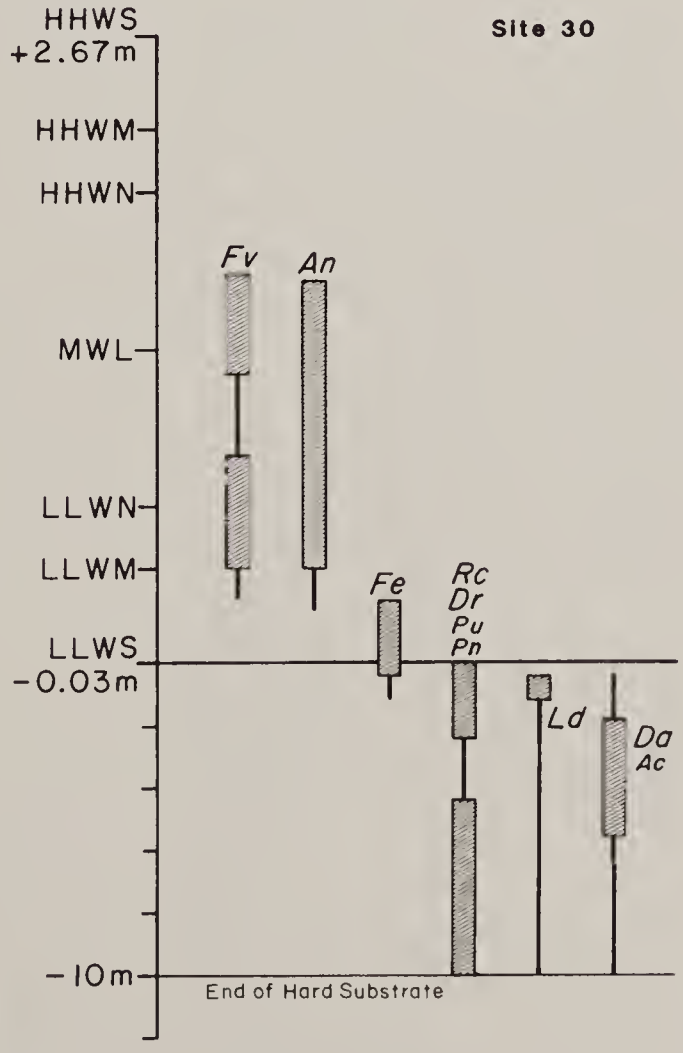

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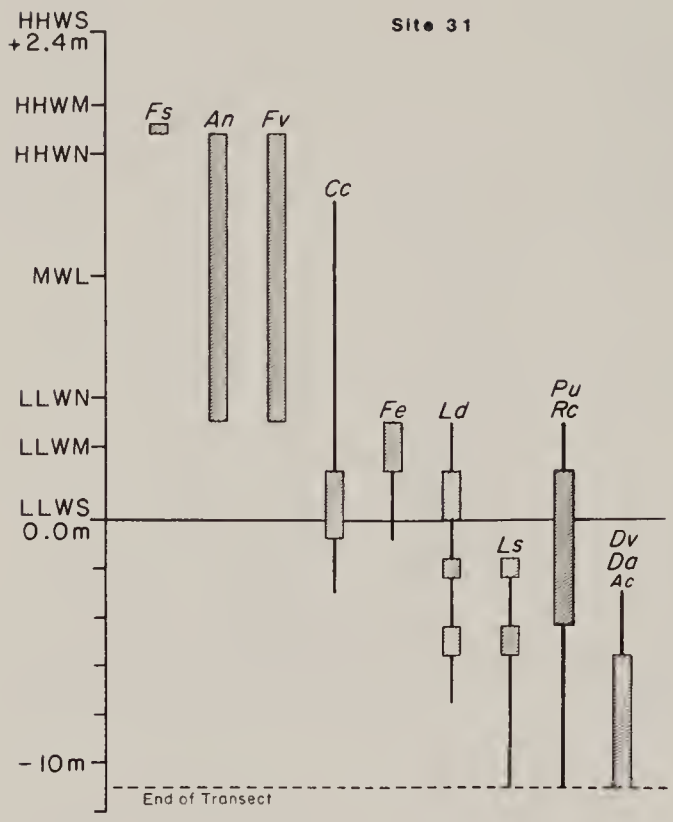

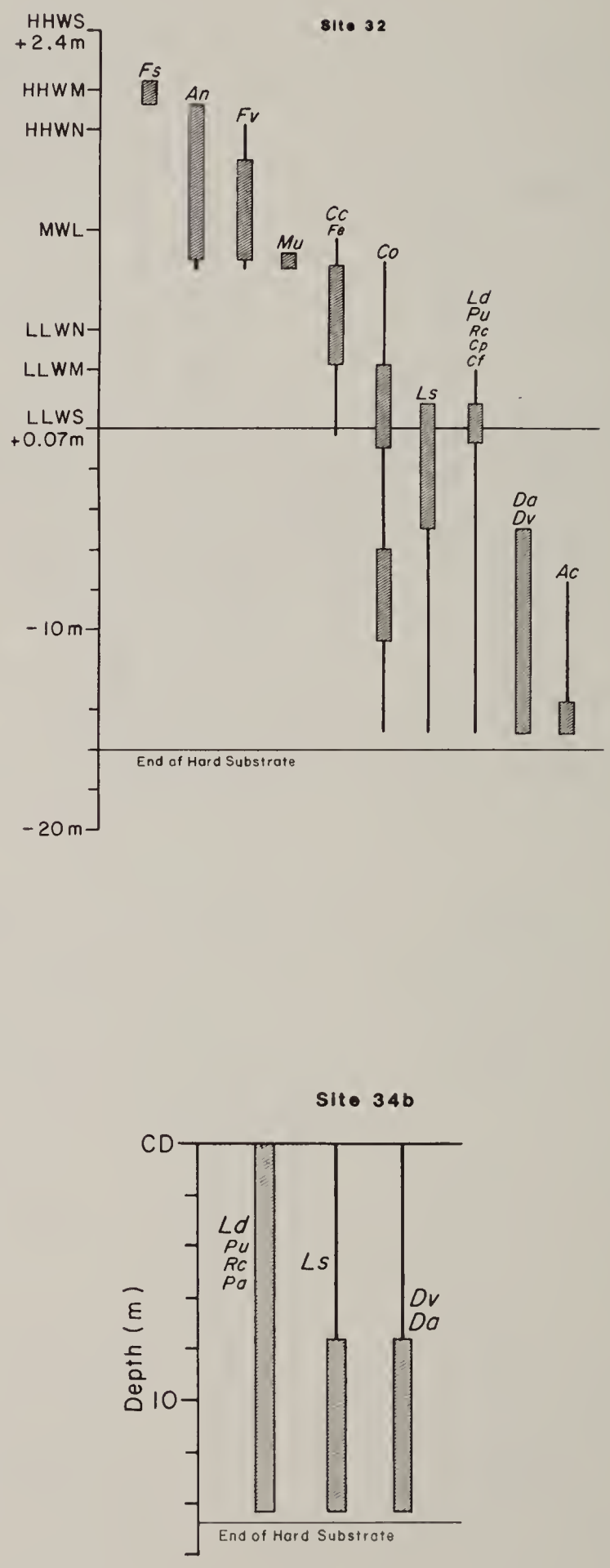

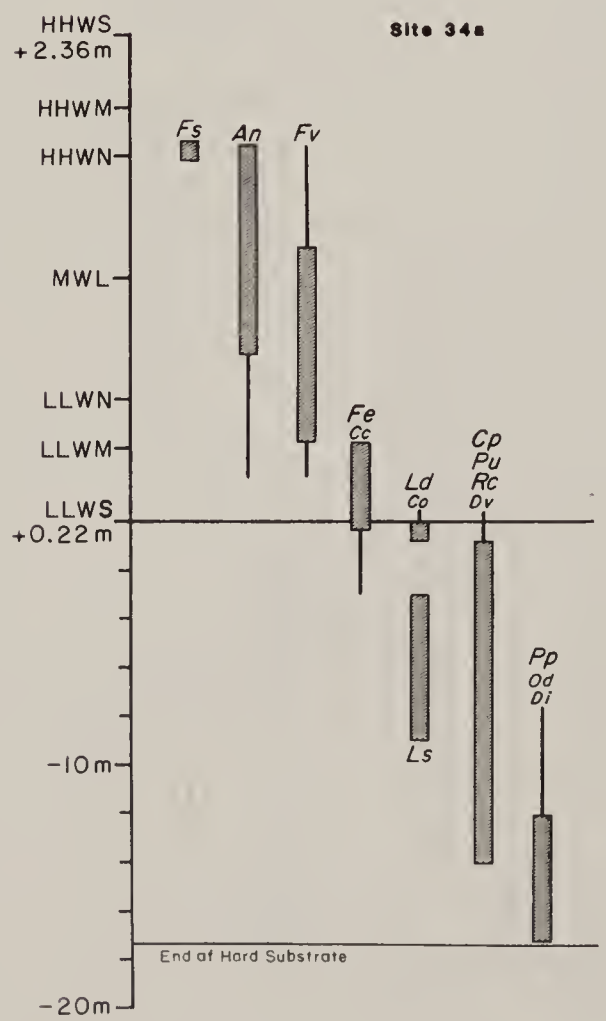

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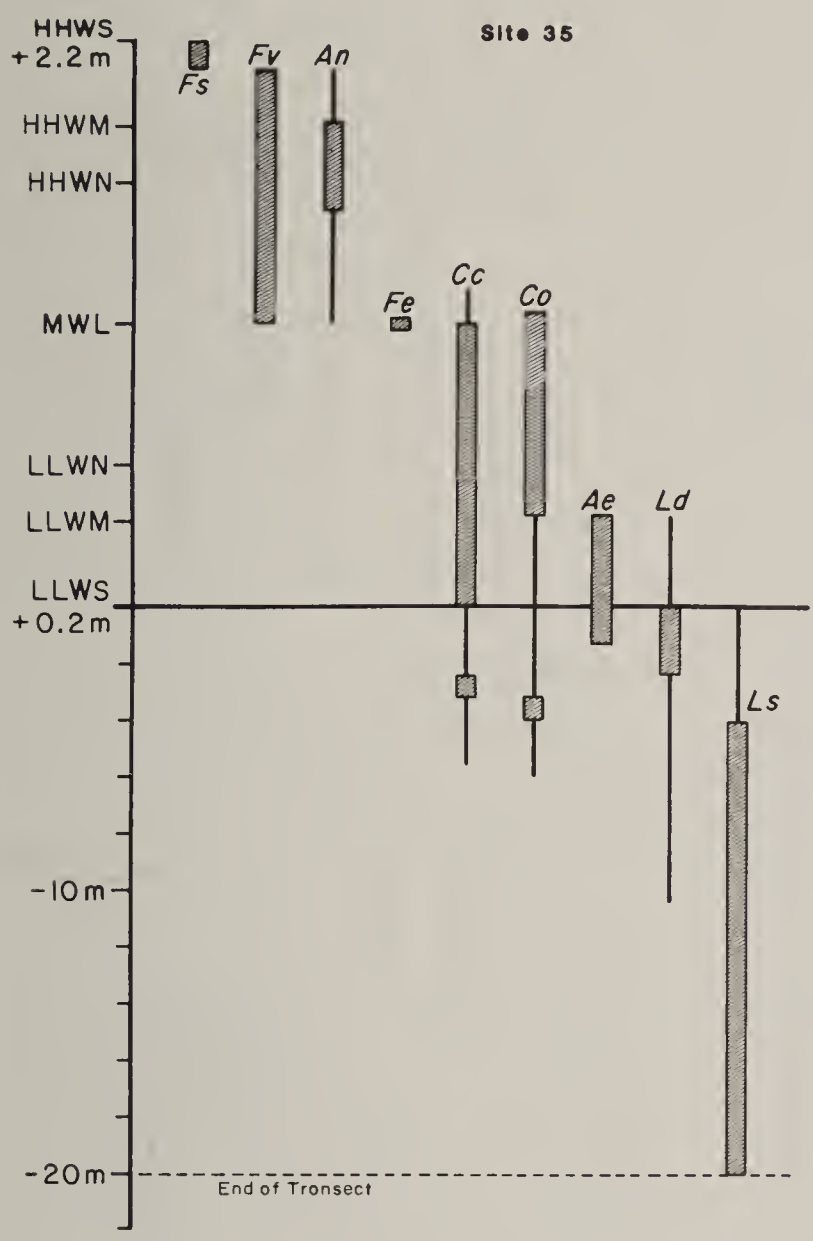

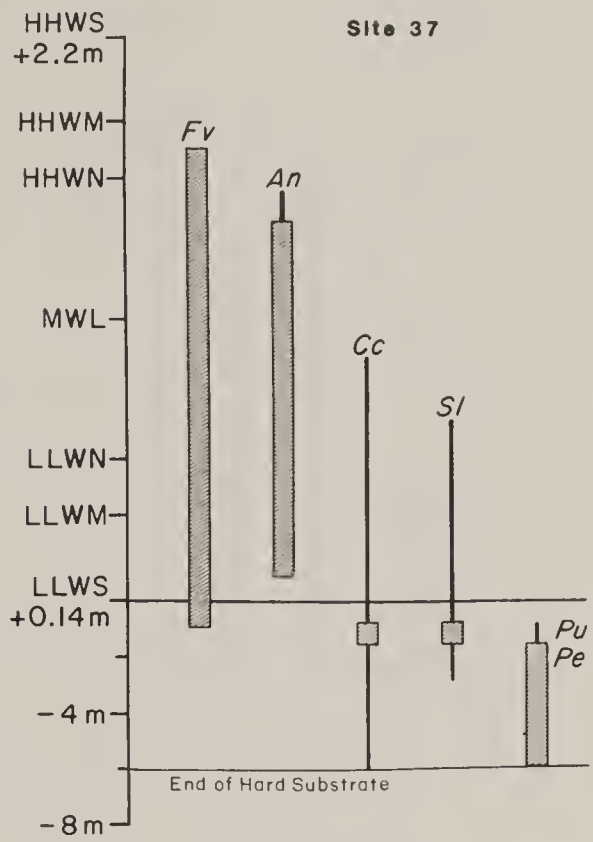

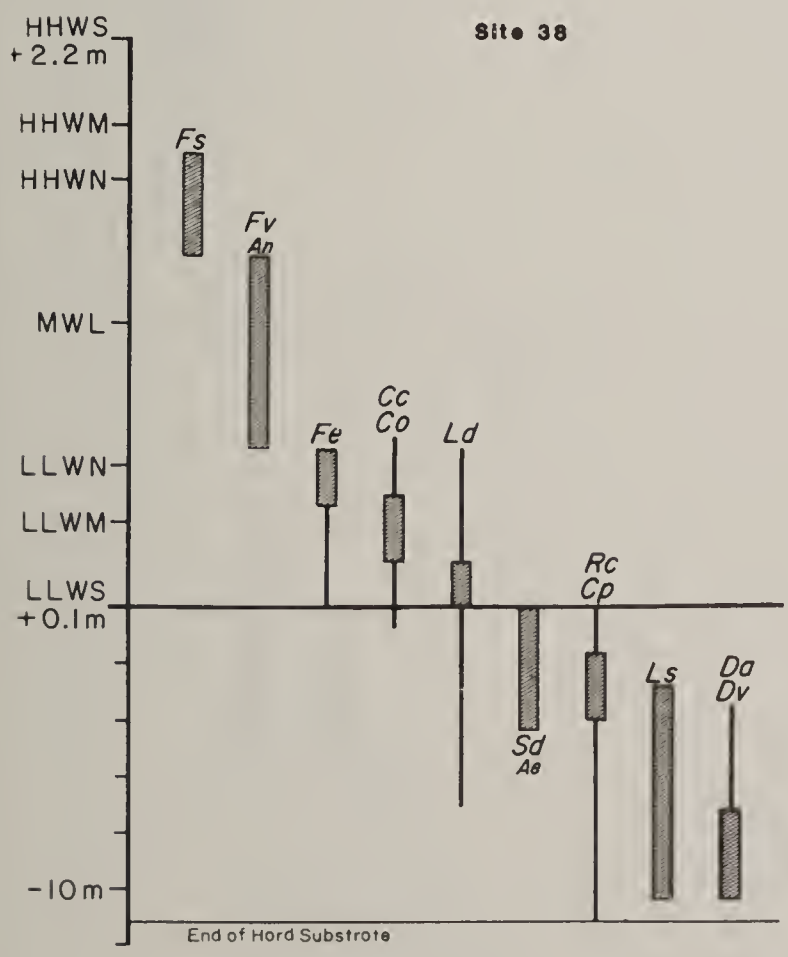

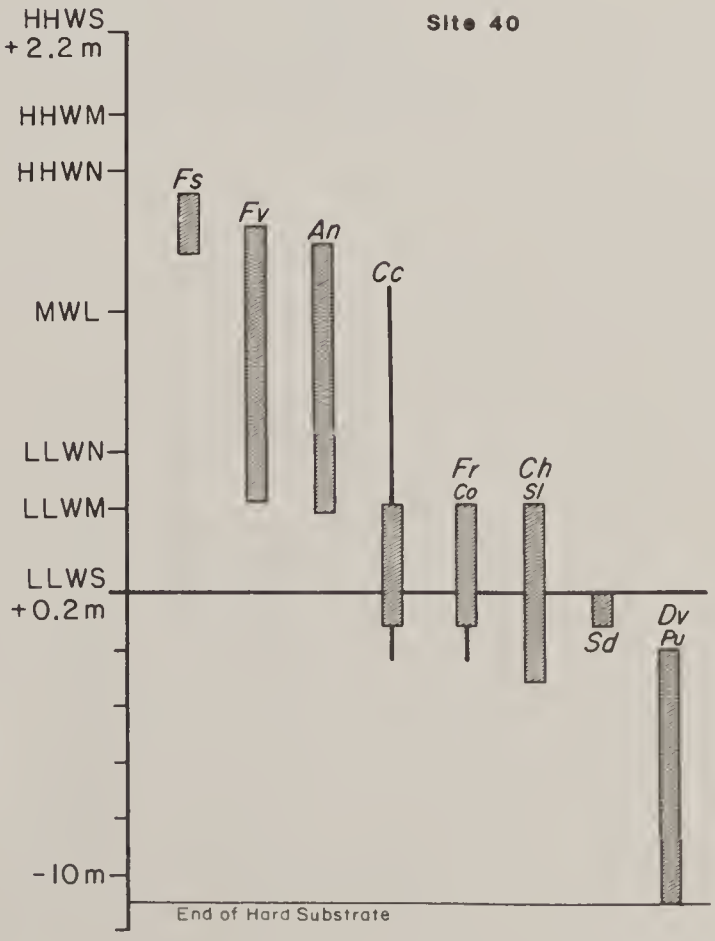

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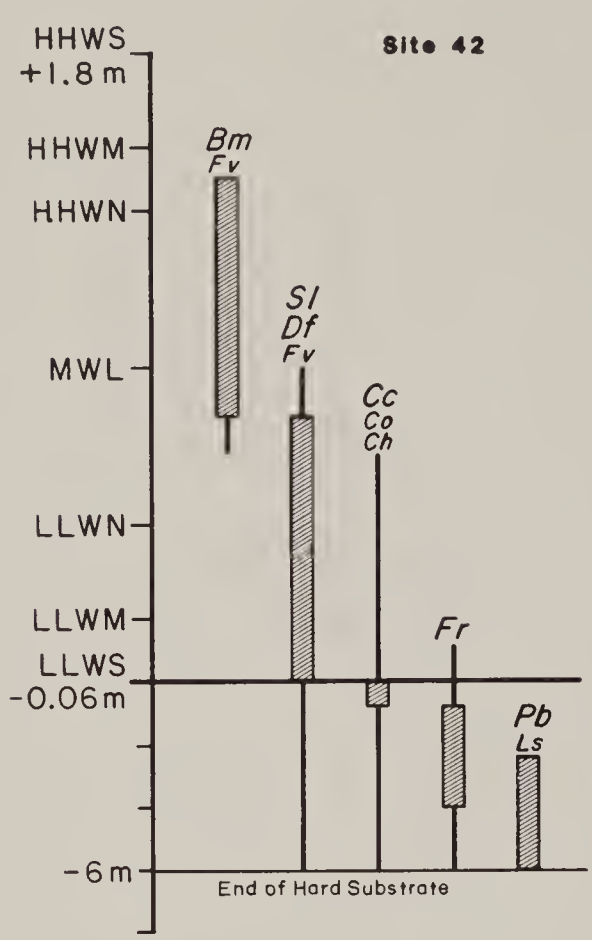

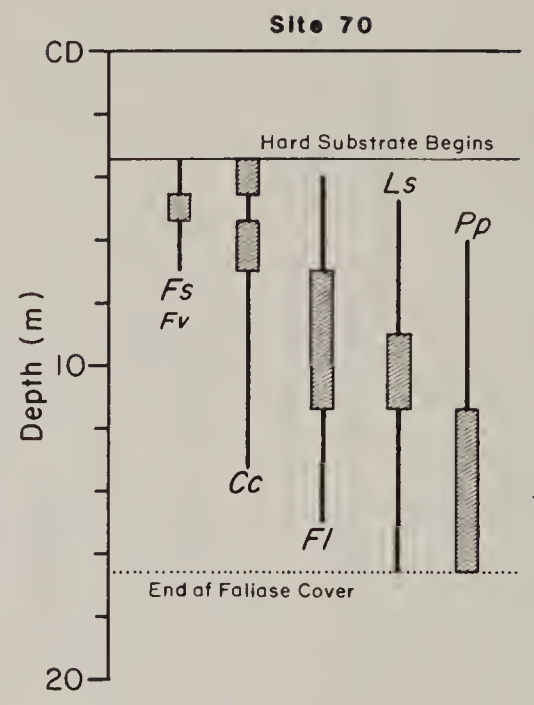

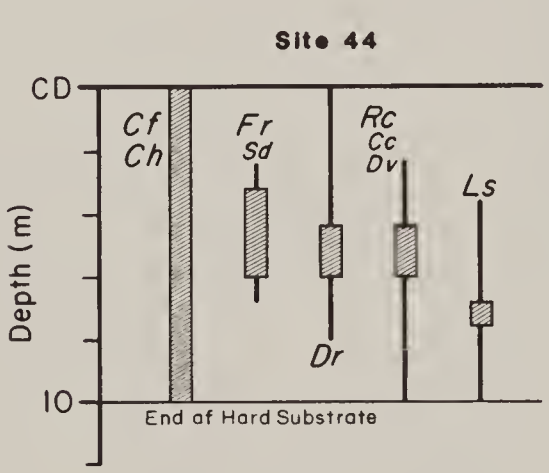

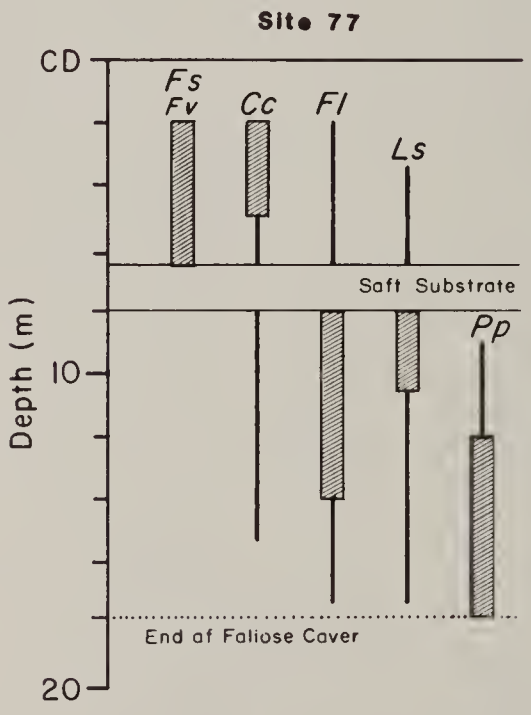

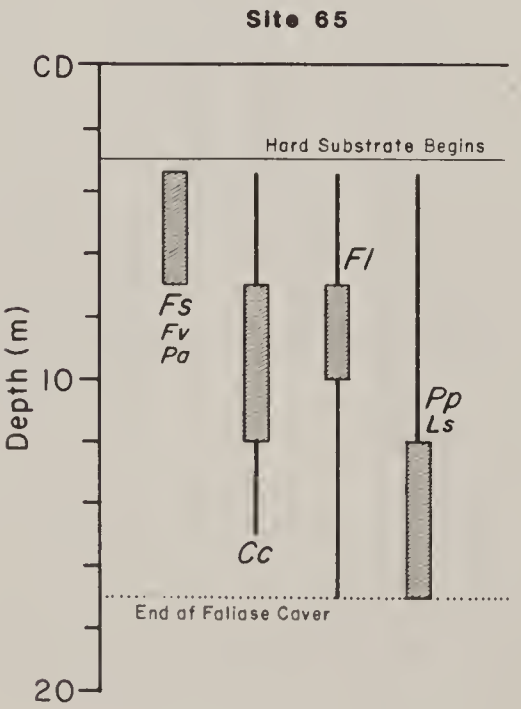

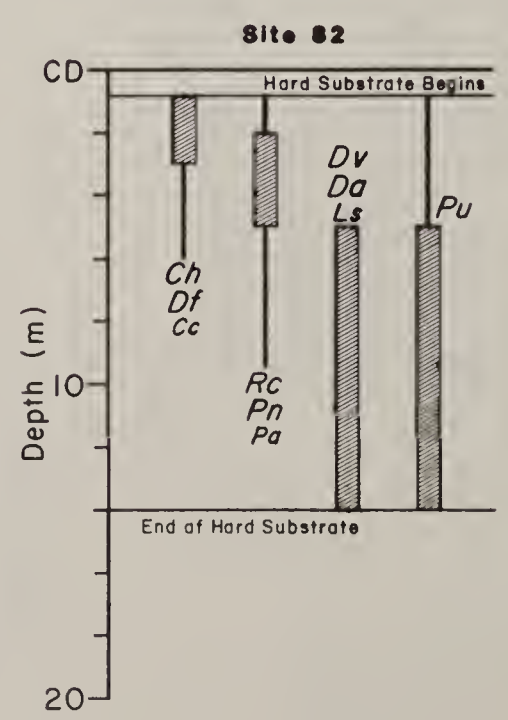

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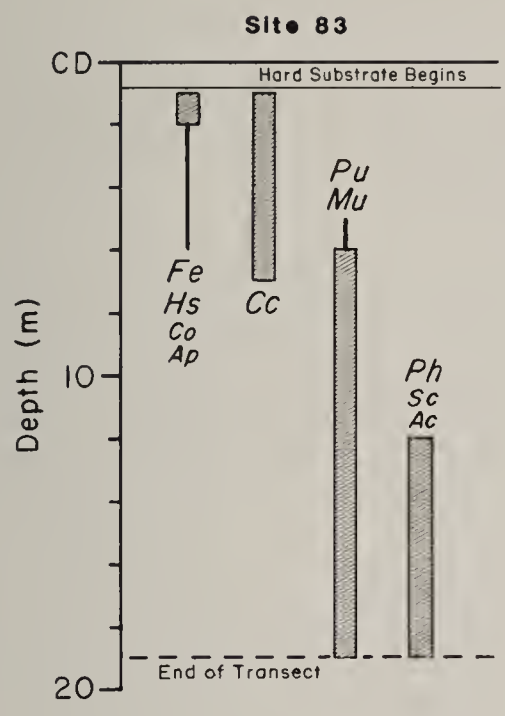

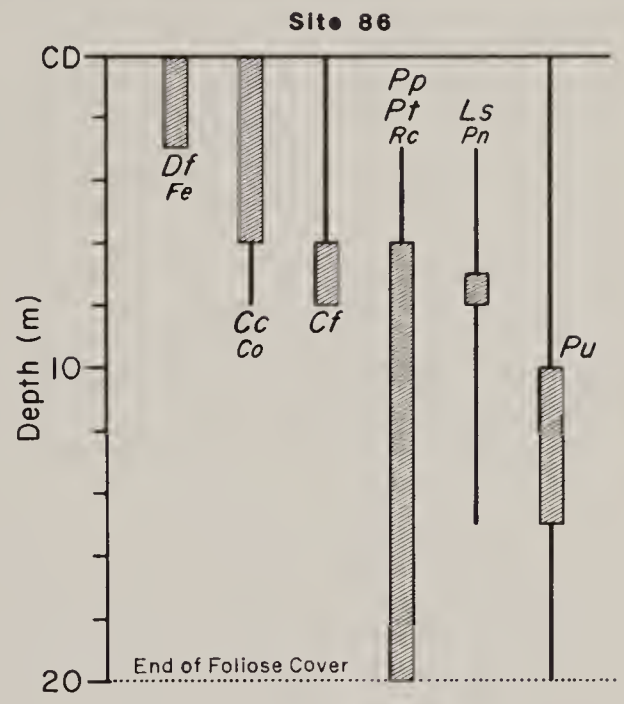

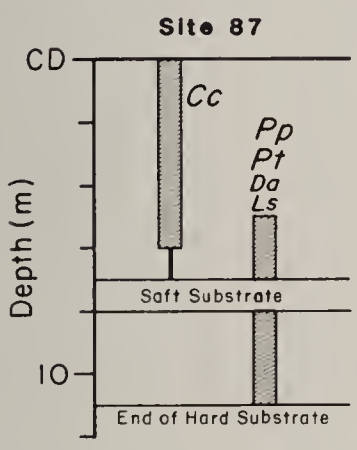

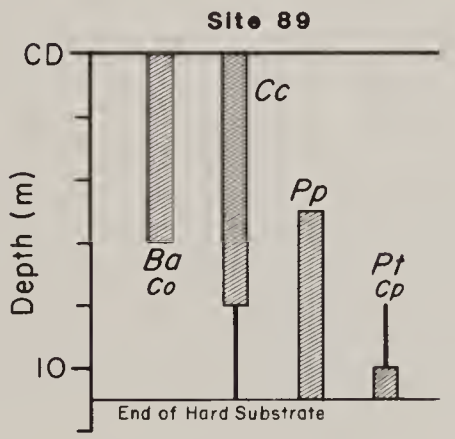

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PURIFYING ALGAL CULTURES - A REVIEW OF CHEMICAL METHODS*

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Chemical methods that have been used to obtain axenic algal cultures are reviewed. The methods include the use of antibiotics, hypochlorite, iodine, hormones, phenol and detergents singly or in combination. Data are given on the use of antifungal agents such as caffeine, griseofulvin and the polyene macrolides e.g. nystatin. When appropriate, the use of physical methods e.g. gel adsorption, sonication, filtration, and osmosis, is described where these techniques are used in conjugation with antifungal/antibacterial compounds.

On a examiné les méthodes chimiques en usage pour la production de cultures axéniques d'algues. Ces méthodes comprennent l'usage d'antibiotiques, de l'hypochlorite, de l'iode, des hormones, du phénol et de détersifs, employés séparément ou en combinaison. Des données sont présentées quant à l'usage de substances antifongiques telles que le caféine, le griséofulvin, et les macrolides de polyene, e.g. le nystatine. L'usage de méthodes physiques, e.g. l'adsorption sur gèle, la sonication, la filtration, et l'osmose, est décrite où on se sert de ces méthodes en conjonction de composés antifongigues ou antibactériennes.

Introduction

Antibiotics and other chemical agents have been used in conjunction with physical methods for many years to obtain axenic cultures of algae. These procedures are widely dispersed in the literature and no systematic attempt has been made to summarize them. The present report draws together this information into a form accessible for scientists and students wishing to purify algae from natural sources. Procedures for isolating algae have been compiled c.f. Stein (1973). Data concerning the source, formula, physical appearance, activity, mode of action and solubility of some of the commonly used antibiotics employed to free algal tissues from bacteria and fungi, can be found in CRC (1980a-e). The taxonomic classification follows that of Bold and Wynne (1978).

Antibacterial Methods Used to Obtain Axenic Cultures

Cyanochloronta - The elimination of bacteria from blue-green algae has proved to be a difficult task. Tchan and Gould (1961), without mentioning the species tested or the concentrations of antibiotics used, reported that none of the following antibiotics eradicated bacteria from *Cyanochloronta;* streptomycin, neomycin, bacitracin, penicillin, chloramphenicol, tetracycline, erythromycin, nystatin, novobiocin, oleandomycin, polymyxin. Combinations of tetracycline and chlortetracycline; penicillin, erythromycin, and aureomycin; and polymyxin, penicillin, erythromycin, and tetracycline were also ineffective. They found that bacteria tested alone were sensitive to some of these antibiotics, but not when associated with the blue-greens, suggesting a protective effect of the algae. Vance (1966) exposed *Microcystis aeruginosa* to several antibiotics in the concentration range 0.01 - 1000 μg mL^{-1}. Only neomycin and dihydrostreptomycin inhibited the growth of bacteria under conditions that allowed algal growth. Four bacteria were isolated; *Xanthomonas oryzae, Brevibacterium insectiphilum, B. linens,* and a small unidentified gram negative rod, and these were sensitive to both antibiotics. A subsequent test employing 1 μg mL^{-1} of neomycin and dihydrostreptomycin singly and together established that only the combination

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produced axenic cultures. However, over the following 6-9 weeks all but two of the axenic cultures on agar slants died. It was discovered that the two survivors were contaminated with a small unidentified gram negative rod, whereas the dead cultures were uncontaminated, suggesting that bacteria may play a role in blue-green metabolism.

Jones et *al.* (1973) used a two-fold dilution series containing a mixture of benzylpenicillin (7-1000 ng mL^{-1}), chloramphenicol (0.3-40 μg mL^{-1}), and neomycin (0.1-16μg mL^{-1}) in an attempt to free *Oscillatoria* sp. from bacteria. With the exception of green-fluorescent pseudomonads, all bacteria were killed. The authors speculated that polymyxin B or one of the newer penicillins e.g. carbenicillin (developed for human *Pseudomonas* infections), kanamycin or colistin might eliminate the pseudomonads.

The use of antibiotics to selectively inhibit contaminants in blue-green algal cultures is not an effective approach because the latter are often as sensitive as the former. This is not surprising because the blue-green algae are prokaryotic (Benson 1985).

Chlorophycophyta - A number of antibiotics used singly and in combination has been applied to green algae to obtain axenic cultures. Galloway and Krauss (1959) tested the potential selective toxicity of terramycin, streptomycin, polymyxin-B-sulfate, tetracycline, penicillin G, bacitracin, chloromycetin, and gramicidin used singly on three species of algae (*Scenedesmus obliquus, Chlorella pyrenoidosa* and *Chlorella vulgaris*), three genera of bacteria (*Flavobacterium sp., Achromobacter sp.* and *Pseudomonas sp.*), and one fungus (*Aspergillus sydowii*). The point at which growth was completely arrested over a period of 2-5 days at 25°C was determined for concentrations ranging from 0.1 to 1000 μg mL^{-1}. Only polymyxin B showed selective toxicity. In an organic medium, >1 mg mL^{-1} was required to halt the growth of *S. obliquus* and *C. vulgaris* whereas $\leq$ 10 μg mL^{-1} arrested the growth of the fungus as well as the bacteria. However, *C. pyrenoidosa* was inhibited at 5 μg mL^{-1}, illustrating the point made by Droop (1967) that even though algae may be closely related, they can exhibit widely different tolerances to antibiotics. Further experiments revealed that 640 μg mL^{-1} of polymyxin B suppressed photosynthesis of *C. pyrenoidosa,* whereas *S. obliquus* and *C. vulgaris* were unaffected. A combination of penicillin and streptomycin applied over several days enabled Berglund (1969) to sterilize multicellular green algae. *Enteromorpha sp.* and *Cladophora gracilis* exposed to 1 mg mL^{-1} and 0.7 mg mL^{-1} for 16 and 17 days, respectively, were freed of all contaminants. Similarly, *Enteromorpha linza* was purified after 8-12 days in 1.2 mg mL^{-1} penicillin and 0.7 mg mL^{-1} streptomycin. However, *Bryopsis plumosa* remained contaminated despite exposure to penicillin, streptomycin, griseofulvin, undecyclenic acid and "Jodopax" alone and in combination. Millner et *al.* (1979) added 35 μg mL^{-1} each of penicillin G and streptomycin sulfate to the enzyme medium they were using to prepare protoplasts from *Enteromorpha intestinalis,* but no mention was made of the sterility of the culture. Gibor and Izawa (1963) sterilized *Acetabularia mediterranea* by washing it thoroughly in sterile seawater and then in a solution containing (per mL) 1 mg streptomycin and penicillin, 200 μg chloramphenicol and neomycin, and 34 μg mycostatin. Later, Green et *al.* (1967) purified *A. mediterranea* with a combination of the antibiotics (per mL) 1 mg penicillin, 200 μg neomycin, and 34 μg mycostatin.

Sterile mixtures of several antibiotics were employed by Jones et *al.* (1973) in four experiments as indicated in Table I, to purify cultures of *Pediastrum boryanum* and *Chlorella vulgaris.* In the first experiment, Series A, a six-tube dilution series was prepared such that each concentration was only half of the previous one. The initial mixture contained (μg mL^{-1}): 2000 benzylpenicillin, 500 tetracycline, 60 chloramphenicol, 50 aureomycin, 400 ceporin, 10 neomycin-SO_4 and 10 streptomycin-SO_4.

Table 1 Concentrations of antibiotics ($\mu g\ mL^{-1}$) in algal cultures of *Chlorella vulgaris* and *Pediastrum boryanum* (adapted from Jones et al. 1973).

	Series A[a] Dilution Tube					
Antibiotic	a	b	c	d	e	f
Benzylpenicillin	2000	1000	500	250	125	62.5
Tetracycline	500	250	125	62.5	31.25	15.6
Chloramphenicol	60	30	15	7.5	3.75	1.9
Aureomycin	50	25	12.5	6.25	3.12	1.55
Ceporin	400	200	100	50	25	12.5
Neomycin-SO_4	10	5	2.5	1.25	0.6	0.3
Streptomycin-SO_4	10	5	2.5	1.25	0.6	0.3

[a] Series B = 2X conc. of Series A minus streptomycin.
Series C = 2X conc. of Series A minus (streptomycin + neomycin).
Series D = 2X conc. of Series A minus (streptomycin + neomycin + ceporin).

Subsequent experiments were carried out at twice the concentrations used in Series A, although with reduced numbers of antibiotics. For example, all of the initial concentrations of antibiotics in Series B were doubled except for streptomycin-SO_4 which was omitted. Similarly, streptomycin and neomycin were excluded from Series C while streptomycin, neomycin and ceporin were dropped from Series D. Two-week-old cultures were incubated for 24 h with antibiotics, whereupon an aliquot was removed and added to Bold's basal medium and grown for two weeks prior to testing for sterility. Sterility was achieved for *P. boryanum* in certain dilution tubes in all four experiments (Series A - tube b; Series B - tubes c and d; Series C - tubes a, b, c, d; Series D - tubes b, c, d). Since sterile cultures were obtained in Series D where only four antibiotics were used, it appears that streptomycin, neomycin and ceporin were of little value in deriving axenic cultures. If streptomycin is largely ineffective in purifying green algal cultures, it should be omitted as Provasoli (1966) reported that it may induce chlorophyll bleaching. It should also be noted that only one tube became bacteria-free in Series A even though all seven antibiotics were present. This may be attributed to the fact that the concentrations in this series were only half of those in Series B, C and D, but it is also possible that there was antagonism occurring between the antibiotics. For example, Speck *et al.* (1951) reported that aureomycin and terramycin antagonized the action of penicillin. With regard to *C. vulgaris,* only tubes b, c, d, e, and f of Series B were rendered sterile. Series A was contaminated with bacteria and yeasts while Series C and D were overrun with yeasts. Apparently the concentrations in Series A were too low to destroy the contaminants, whereas in Series C and D the elimination of bacteria allowed the opportunistic yeasts to proliferate. Provasoli *et al.* (1951) described the same phenomenon where yeasts predominate in the absence of previously established bacteria and he noted that a fungicide would be required to obtain sterile cultures (see below). However, it should be pointed out that neomycin was absent from Series C and D. It was hypothesized that it had played a role in eradicating yeasts from Series B.

A different approach was tried by Provasoli (1958), who placed pieces of *Ulva* sp. thallus on the surface of an agar medium containing a mixture of antibiotics. He found that 120 μg of penicillin together with 1 μg each of chloramphenicol, neomycin, and polymyxin B per mL of agar were sufficient to kill all bacteria and 66% of the diatoms. The bacteria-free algal cultures developed into atypical, short filamentous germlings. Provasoli (1958) postulated that this effect was due to the absence of bacteria and their beneficial effect on the gross morphology of the *Ulva.* Antia and

Kalmakoff (1965) employed a combination of antibiotics together with a non-ionic surfactant, Tween-80 (Brown and Bischoff 1962), to purify *Tetraselmis maculata*. Penicillin (100 μg mL^{-1}), streptomycin (50 μg mL^{-1}), chloramphenicol (25 μg mL^{-1}), and Tween-80 (20-40 μL mL^{-1}) proved to be successful.

Euglenophycophyta - Pappas and Hoffman (1952) tried to free *Euglena gracilis* of bacteria using penicillin (120 μg mL^{-1}), dihydrostreptomycin (1 mg mL^{-1}), and aureomycin (400 μg mL^{-1}) singly, in pairs, and in combination over a 10 day period at 23°C. Success was achieved only when all three antibiotics were combined. Later, Ebringer (1964) found in 14-day exposures that the following concentrations of antibiotics (μg mL^{-1}) tested individually could produce permanent bleaching of the chloroplasts: erythromycin (200); streptomycin (200); kanamycin (500); viomycin (500); carbomycin (500); and spiramycin (2000).

Chrysophycophyta - Chrysophyceae - Pintner and Provasoli (1968) derived axenic cultures of three species of *Chrysochromulina* using two different antibiotic combinations. *C. strobilus* was rendered axenic following treatment for an unspecified period with a solution of AM9 antibiotics in the concentration range 0.06 - 0.1 mL mL^{-1}. AM9 contained (μg mL^{-1}): 20 polymyxin B, 6600 dihydrostreptomycin, 260 tetracycline, 560 chloramphenicol, 3000 penicillin G, and 400 neomycin. The media of the remaining species, *C. kappa* and *C. brevefilum*, were treated with kanamycin (100 μg mL^{-1}) and pencillin (3-6 mg mL^{-1}). Samples were transferred daily to media without antibiotics until bacteria-free cultures were obtained.

Bacillariophyceae - One of the first published studies on purifying diatom cultures involved the use of penicillin and streptomycin singly and in combination. Spencer (1952) exposed *Nitzschia closterium* forma *minutissima* to concentrations of penicillin ranging from 0.03-6 mg mL^{-1} for 16 days and found that the highest concentration in which the diatom survived (0.6 mg mL^{-1}) also permitted bacterial growth. Streptomycin inhibited diatom growth at 0.7 mg mL^{-1} but failed to rid the culture of bacteria. Combinations of both penicillin (0.03, 0.06 and 0.03 mg mL^{-1}) and streptomycin (0.07, 0.13 and 0.7 mg mL^{-1}) over 16 days also were unsuccessful.

A different approach was tried by Berland and co-workers in which bacteria were isolated from planktonic algal cultures and identified. Their susceptibility to a number of antibiotics tested singly was evaluated and compared to that of five species of diatoms. Berland *et al.* (1969) isolated 25 species of bacteria from cultures of *Asterionella japonica, Chaetoceros lauderi, Lauderia borealis, Phaeodactylum tricornutum, Leptocylindricus danicus,* and *Stichochrysis immobilis*. Berland and Maestrini (1969a) tested susceptibility of the diatoms to aureomycin, terramycin, penicillin, kanamycin, neomycin, streptomycin, dihydrostreptomycin, chloramphenicol, and polymyxin B while incubated for one week at room temperature. The effectiveness of each antibiotic was judged relative to its toxicity to the diatoms tested: *A. japonica, C. lauderi, L. danicus, P. tricornutum* and *Rhizoselenia fragillissima*. Aureomycin, terramycin, chloramphenicol, and polymyxin B were not selectively toxic (Table II), because their minimum inhibitory concentrations (MIC) towards the diatoms after a 6-day exposure at 18°C were in the ranges (μg mL^{-1}) 35-100, 5-20, 6->80 and 0.75-55, respectively (Berland and Maestrini 1969b). The MICs (μg mL^{-1}) of kanamycin (250->1000), neomycin (100->1500) and streptomycin (550->1000) to the diatoms tested suggest that, in most cases, the antibiotics are more toxic to bacteria, with the exception of *Flavobacterium* sp. and *Xanthomonas* sp., than to diatoms. By contrast, penicillin with MICs of 700->1000 μg mL^{-1} was more effective against *Flavobacterium* sp. and *Xanthomonas,* but less effective against pseudomonads. No attempt was made to sterilize the diatoms with dihydrostreptomycin so its selective toxicity could not be evaluated.

Table II Minimum inhibiting concentrations ($\mu g\ mL^{-1}$) of antibiotics for bacteria isolated from planktonic algal cultures (from Gerland and Maestrini 1969a).

Genera and Species	Aureomycin	Terramycin	Penicillin	Kanamycin	Neomycin	Streptomycin	Dihydro-streptomycin	Chloram-phenicol	Polymyxin B
Pseudomonas									
P. aestumarina	300	200	4000	200	20	>10000	>5000	100	>900
P. cruciviae	60	50	1	30	30	30	25	2	4
P. marinoglutinosa	400	1000	250	400	100	400	1000	150	15
P. (marinoglutinosa)	300	1500	1500	40	40	30	20	25	10
P. riboflavina	50	20	50	20	20	20	75	25	20
P. stereotropis	80	175	0.25	200	50	150	25	2	25
P. sp. 1	200	700	2000	50	60	30	25	15	50
P. sp. 2	200	750	750	70	50	200	200	7.5	10
P. sp. 3	40	100	12	200	30	100	>1000	1.5	>900
Vibrio									
V. algosus	400	150	1750	400	200	300	200	10	5
V. phytoplanktis	300	175	15	400	175	80	150	1.5	10
Agarbacterium									
A. mesentericus	200	1500	3000	100	30	20	60	5	50
Xanthomonas									
X. sp. 1	100	100	30	100	1500	>10000	>5000	1	500
Achromobacter									
A. parvulus	80	150	1	200	125	>10000	200	2	500
A. stationis	15	50	0.25	7	10	15	15	1.5	50
A. stenohalis	50	20	50	60	15	5	10	1.5	2
A. sp. 1	200	750	750	50	30	40	25	2	7.5
Flavobacterium									
F. aquatile	200	125	50	>5000	>1000	>10000	>5000	30	>1000
F. lutescens	300	75	100	>5000	>2100	10000	9500	75	>1000
F. peregrinum	30	100	2	30	10	7.5	5	5	150
F. sp. 1	30	40	0.05	80	30	>100000	125	0.75	2
F. sp. 2	300	100	750	>5000	>1000	>100000	>5000	100	>1000
F. sp. 3	400	150	400	60	>2100	100000	9500	200	600
Micrococcus									
M. sp. 1	50	250	15	80	400	25	100	2	150
Staphylococcus									
S. aureus	40	150	7	9	30	20	20	5	400
S. aureus peni (-)[a]	20		150						
S. aureus peni. (+)			>1000						

[a] *S. aureus* penicillin (-) and *S. aureus* penicillin (+) are collection strains from the "Faculté de Pharmacie de Marseille", France.

This type of study points out the large differences in susceptibility to the same antibiotic which can occur between species of the same genus. For example, the MIC of penicillin for *Pseudomonas aestumarina* was 4 mg mL^{-1} whereas for *P. stereotropis* is was only 0.25 μg mL^{-1}. Because of these variations and those of the diatom species themselves, treatment with a single antibiotic would be unlikely to render a culture axenic.

Droop (1967) reported that a short exposure (24 h) to a high concentration of a combination of antibiotics would eliminate bacteria. He tested two separate mixtures for diatoms as indicated in Table III. The original concentration (Table III) was decreased by a factor of two through each of six successive subcultures before deleting the antibiotics. In a "typical" experiment an intermediate concentration was usually successful in eliminating the bacteria while yielding a viable alga. Jones et *al.* (1973) tried Droop's mixture 1 (Table III) on *Nitzschia capitellata* and *Amphora but a 24 h exposure bleached the chloroplasts. The mixture was then modified (2 mg* mL^{-1} penicillin, 1.6 mg mL^{-1} ceporin, and 200 μg mL^{-1} aureomycin) and, after a "short exposure", the cultures were rendered axenic. No alteration in growth, pigmentation or cytology in either species occurred. However, using the same treatment as for green algae described above, Jones et *al.* (1973) were unable to sterilize *Tribonema viride* Pasch., due to a heavy growth of *Pencillium* spp. The addition of a fungicide such as nystatin might overcome this problem (see below).

Table III Concentrations of antibiotics used in mixtures to sterilize diatom cultures (adapted from Droop 1967).

Antibiotic	Mixture 1 (μg mL^{-1})	Mixture 2 (μg mL^{-1})
Benzylpenicillin	8000	8000
Streptomycin-SO_4	1600	1600
Chloramphenicol	200	80
Neomycin	-	400

Pyrrhophycophyta - Divan and Schnoes (1982) exposed four "red tide" organisms *Gonyaulax catenella, G. excavata, G. tamarensis* subsp. *excavata,* and *G. tamarensis* Plymouth to a number of antibiotics in attempts to produce an axenic culture. Actively growing cultures were inoculated into seawater medium together with amphotericin B, chloramphenicol, dihydrostreptomycin sulfate, neomycin sulfate, polymyxin B sulfate, potassium penicillin G, and streptomycin sulfate singly and in combination. After 16-17 days of growth at 15°C, the cells were centrifuged and resuspended in medium prior to being examined microscopically for changes in size, morphology and motility. In general, *G. catenella* and *G. excavata* tolerated dihydrostreptomycin, neomycin, penicillin, and streptomycin applied singly over a concentration range of 10-1000 μg mL^{-1}. The other algal species were susceptible to all the antibiotics tested. Amphotericin B and chloramphenicol inhibited dinoflagellate growth in all four species. For example, 25 μg mL^{-1} of amphotericin B inhibited the growth of both *G. catenella* and *G. excavata* while 10 μg mL^{-1} completely arrested the growth of *G. tamarensis* subsp. *excavata* and *G. tamarensis* Plymouth. Chloramphenicol at concentrations >50 μg mL^{-1} caused cell lysis in all species. Five antibiotics with the lowest toxicity to the dinoflagellates (dihydrostreptomycin, penicillin G, polymyxin B, streptomycin, and neomycin) were then evaluated for bactericidal activity. No single antibiotic was completely effective. The group that yielded axenic cultures of *G. catenella* and *G. excavata* consisted of dihydrostreptomycin, neomycin, penicillin G, and amphotericin B at concentrations of 250, 250, 500 and 5 μg mL^{-1},

respectively. This combination did not reduce growth, motility or toxin production in the dinoflagellates. Cultures of *G. tamarensis* subsp. *excavata* and *G. tamarensis* Plymouth could not be rendered axenic as concentrations of antibiotics necessary to eradicate the bacteria were toxic to the dinoflagellates themselves.

Phaeophycophyta - Saga and Sakai (1982) described a method for deriving pure cultures of the macroscopic alga *Dictyosiphon foeniculaceus,* which had been treated with an antibiotic mixture consisting of (mg mL^{-1}): penicillin G (125), streptomycin sulphate (50), and chloramphenicol (20). The alga was wiped with gauze, rinsed in sterile seawater and cut into one cm lengths, whereupon ten sections were introduced into the antibiotic solution and maintained at 5°C for two days. After rinsing in sterile medium, zoospores were collected and spread over the surface of an agar plate. One month later, 0.5 to 1.0 mm masses of filamentous thalli had grown. Those which appeared sterile were transferred to test tubes containing ASP 12-NTA medium (Provasoli 1963) and monitored for cloudiness after growth for an additional month. Using this method, more than 90% of the sections were reported axenic as were some strains of other algae that were tested (e.g. *Pogotrichum yegoense, Ulva pertusa* and *Laminaria angustata).*

Saga and Sakai (1983) also developed axenic cultures of *L. angustata* using a procedure without antibiotics. Sporophytes were cut into 5 cm lengths, wiped with gauze and rinsed several times in sterile seawater, whereupon both ends were dipped in absolute ethanol, burned and cut off with a sterile knife. The remaining tissue was punched axially using a sterile cork borer and the medullary tissue was sectioned approximately 2 mm thick, prior to being inoculated on 50 mL ASP 12-NTA medium solidifed with 1.0% agar. A sterility test using ST3 medium (Provasoli et al. 1957) revealed that all tissues were axenic.

Rhodophycophyta - *Goniotrichum elegans* was freed from bacterial contaminants by treating small pieces of tissue for 12-14 days with penicillin (0.6 mg mL^{-1}), and streptomycin (0.7 mg mL) (Fries 1963). Similarly, *Asterocytis ramosa* was rendered axenic by a three-day treatment in streptomycin (0.7 mg mL^{-1}) followed by eight days in pencillin (4.8 mg mL^{-1}). However, other unidentified species were not freed of contaminants using only these antibiotics. In addition, the combination of antibiotics used by Provasoli (1958) to obtain axenic cultures of *Ulva* was tested on *Nemalion multifidum, Antithamnion plumula,* and *Ceramium strictum* without success. Similarly, Cheney *et al.* (1986), using a modified AM9 combination were unable to free *Gracilaria lemaneiformis* from bacteria after a 24-48 h exposure. Bradley et al. (1988), however, did report some success with small pieces of tissue exposed to discs impregnated with antibiotics on agarose plates. Unialgal cultures of *Agardhiella subulata, G. lemaneiformis,* and *Porphyra yezoensis* were separately and aseptically chopped into 1 to 3 mm pieces and spread evenly across agarose solidified media in petri dishes. A variety of antibiotic discs was then placed on the agar surface. Following incubation at 22°C for three to five days, healthy pieces of tissue were removed from clear zones, where the bacteria were inhibited, and placed on other agarose plates to test for sterility. After a further five to seven days at 22°C, the pieces that still appeared "clean" were transferred to new medium and regenerated into whole plants. In a typical experiment, six of twenty-four antibiotic discs would produce clear zones. Of the ten pieces of tissue from the polymyxin B and erythromycin zones, only one of each proved to be axenic and grew into mature plants. This method also yielded axenic cultures of *Porphyra yezoensis, Enteromorpha linza* and *Laminaria japonica.* However, *A. subulata* did not survive longer than five days unless plant growth regulators were added. A concentration of α-naphthaleneacetic acid (0.01 mg L^{-1}) and zeatin (0.01 mg L^{-1}) ensured survival for ten days. In one experiment four axenic pieces were obtained from the rifampicin zone while one was recovered from the erythromycin zone.

The use of antibiotics can have adverse sublethal effects on the physiology of algae. Sheath (1975) reported that chloramphenicol and cycloheximide (see below) at concentrations of 100 μg mL^{-1} reduced the rate of photosynthesis in *Porphyridium purpureum* by about 50% and 75%, respectively. In addition, starch accumulation was prevented by rifampicin (an inhibitor of RNA polymerase) when added to *P. purpureum* after transfer of the alga from the dark to the light.

Antibiotics Used in Conjunction with Osmotic Methods

Chlorophycophyta - Brown (1982) developed a method of freeing algal cultures of bacteria based on the principle that bacterial cells treated with antibiotics at a high salinity will accumulate high concentrations of osmotic solutes and have fragile cell walls which, when abruptly changed to a hypotonic medium, would lyse, leaving the more sturdy eucaryotic algae to survive and become axenic. Artificial seawater (primary salts - 478 mM NaCl, 25.6 mM $MgCl_2$, 28.9 mM $MgSO_4$, 10.5 mM $CaCl_2$, 10.4 mM KCl, 2.4 mM $NaHCO_3$ and 97 μM H_3BO_3) with the addition of f/2 nutrients less silicate (Guillard and Ryther 1962) was used to grow marine unicells consisting of two strains of *Nanochloris* (Univ. Washington isolate 20-2-2 and Utex 2055), *N. oculata* (Utex 2164) and two freshwater species, *Mesotaenium caldariorum* (Utex 41) and *Chlorella sp.* (Univ. W. Ont. isolate 56) at 20°. The two *Nanochloris* spp. were grown in 200% primary salts until the exponential stage of growth, when an antibiotic mixture consisting of penicillin G. (2 mg mL^{-1}), ampicillin (2 mg mL^{-1}), carbenicillin (1 mg mL^{-1}), cycloserine (100 μg mL^{-1}), and vancomycin (300 μg mL^{-1}) was added and the culture was incubated for 72 h. The cells were then centrifuged, resuspended in 7% seawater and subjected to 2 min of ultrasonic treatment. The others were treated in a similar manner except that initially they were grown to the exponential phase in 100% seawater (*Chlorella sp.* and *N. oculata*) or 7% seawater (*M. caldariorum*), whereupon they were centrifuged and resuspended in sterile 200% seawater with antibiotics. Both controls and antibiotic-treated cells were washed, centrifuged and resuspended in 7% seawater. The resulting algal cultures were tested for contamination by inoculation into three types of medium as well as by light and TEM microscopy. All of the algae tested, with the exception of *M. caldariorum*, survived the treatment. However, only two cultures became axenic; both were *Nanochloris sp.* In one case, 75% of the cultures were axenic, whereas, in the other only 33% were free of bacteria.

Brown (1982) pointed out that all of the antibiotics used were bacterial cell wall inhibitors designed to work together with osmotic shock and sonication to lyse bacterial cells. He discouraged the use of commonly recommended bacteriostatic agents such as streptomycin or chloramphenicol in combination with cell wall inhibitors as they render the inhibitors ineffective (Tipper and Wright 1979).

Antibiotics Used With Hypochlorite Treatments

Phaeophycophyta - Druehl and Hsiao (1969) exposed four species of brown algae, *Nereocystis luetkeana, Laminaria saccharina, Costaria costata* and *Alaria marginata* to a mixture of antibiotics consisting of penicillin G (620 μg mL^{-1}), streptomycin sulfate (250 μg mL^{-1}), chloramphenicol (100 μg mL^{-1}), and/or a 1% solution of sodium hypochlorite in order to obtain axenic cultures. Mature sori were cut from the sporophyte, wiped off and rinsed several times in sterile seawater whereupon they were immersed in the antibiotic mixture for 3 h, followed by 20-30 min in NaOCl. The sori were aseptically trimmed, rinsed 5 times in sterile ASP 2M medium (Druehl and Hsiao 1969) and placed in the medium. Meiospores, released usually within 2 h, were transferred to culture tubes containing sterility test medium and examined over a 10 week period for the presence of bacteria. All species tested became axenic except *C. costata*. Under axenic conditions, the percentage germination of the meiospores and the development of the gametophytes were the same as those of the controls.

However, the sporophyte development was delayed in *A. marginata* and absent altogether in the other species. No explanation was given for this phenomenon, except that the contaminants may have produced a factor necessary for sporophyte development (see below).

Antibiotics Used With Agar Cleaning

Rhodophycophyta - Several antibiotic mixtures consisting of various combinations of chloramphenicol, neomycin, polymyxin, tetramycin, novobiocin, erythromycin, penicillin, streptomycin, and magnamycin were tried unsuccessfully by Tatewaki and Provasoli (1964) to treat three species of *Antithamnion*. They then turned to the "dip and drag" technique of pulling a small thallus piece through 20 mL of agar to which 0.5-1.5 mL of antibiotic mixture had been added. Some success was achieved by washing the thallus 15 times in agar followed by 6 washings in liquid medium and antibiotics. The antibiotic mixture contained (μg mL^{-1}): penicillin (600), dihydrostreptomycin (100), chloramphenicol (50), neomycin (50), polymyxin (6), and tetracycline (21).

Iwasaki (1965) adapted the Tatewaki and Provasoli (1964) method in efforts to isolate axenic tissues of *Porphyra tenera*. An antibiotic mixture containing 24 mg of penicillin and 20 mg of streptomycin was added to 20 mL of agarized enriched seawater and young buds 1 mm long were placed on the surface before being dragged inside the agar. Following each washing, the thallus was brought to the surface and the adhering agar was removed. This procedure was repeated seven times, whereupon the tissue was left overnight in the agar and then washed an additional five times before being transferred to sterile liquid medium. After one month the tissue was transferred to new medium and kept for two months prior to being examined microscopically for bacteria. Of the ten original buds, four were axenic, but only one survived and grew. Recently, Cheney *et al.* (1986) rendered *Gracilaria tikvahiae* axenic using a modification of this method.

Chlorophycophyta - Several species of *Oedogonium* were freed of bacteria by the agar "drag" method of Machlis (1962). Vegetative filaments were washed with distilled water and sterile medium and then dragged through 0.7% agar, followed by three successive plates of 0.7% agar containing 1.5 mg mL^{-1} of penicillin. The filaments were left embedded for 24 h and then transferred to culture tubes of medium without agar. Roughly 40-50% of the filaments became axenic using this technique.

Antibiotics Used With Filter Washing

Pyrrhophycophyta - Soli (1964) devised a system whereby *Peridinium trochoideum* was caught on a filter disc and washed with sterile seawater to remove loosely adhering bacteria. This was followed by a 5 h exposure to a combination of penicillin, streptomycin, polymyxin B, and neomycin, each at a concentration of 30 μg mL^{-1}. Then the sterile seawater was again flushed through the system, leaving the dinoflagellate axenic.

Antibiotics Used with Iodine

Rhodophycophyta - Fries (1963) reported that *Nemalion multifidum* was freed of bacteria by initial treatment with penicillin and streptomycin followed by immersion in a 0.125% molecular iodine solution for an unspecified length of time. As there were still small "unknown particles" adhering, the tissue pieces were inoculated onto agar plates and the plates inverted. Small axenic tips were excised as the alga grew through the agar towards the light.

Antibiotics Used with Iodine and Sonication

Rhodophycophyta - Gibor et al. (1981) have outlined a three-step procedure for obtaining axenic cultures: 1) ultrasonic treatment up to several minutes to eliminate

most epiphytes, 2) immersion in 1% w/v betadine (iodinated polyvinyl pyrrolidone): seawater up to five minutes, and 3) immersion in a solution of antibiotics in unspecified enriched seawater for one week. Using this procedure sterile tissues of *Porphyra lanceolata, Eucheuma uncinatum, Antithamnion* sp., *Gelidium nudifrons,* and *Gracilaria* sp. were established. Polne et *al.* (1980) found that ultrasound was effective in removing epiphytes from several species of algae including *Gelidium robustum, G. nudifrons, Gastroclonium coulteri,* and *E. uncinatum.* The algae, encrusted with diatoms, filamentous red and brown algae and bacteria, were immersed in seawater, placed in the ultrasonic bath for two minutes, removed and resuspended in sterile seawater. This procedure, repeated twice, removed most of the epiphytes except where they were well established. Gibor et *al.* (1981) noted that if some epiphytes remained, they might also survive the betadine and antibiotic procedures, thereby resulting in bacteria-free cultures that were not unialgal. in a later study, Polne-Fuller and Gibor (1984) cut discs of tissue from *Porphyra perforata,* sonicated them in sterile freshwater for 30 s, rinsed them in sterile seawater and repeated the procedure twice using sterile seawater. The discs were then immersed in a 1% betadine solution for 7 min and rinsed in sterile seawater. After treatment in a 1:10 dilution of the following antibiotic mixture (1 g penicillin G, 2 g streptomycin, 1 g kanamycin, 25 mg nystatin, 200 mg neomycin in 100 mL distilled water) the tissues were described as axenic.

Antibiotics with Hormones and Tissue Excision

Rhodophycophyta - Using medullary tissue, Chen and Taylor (1978) were able to render *Chondrus crispus* axenic. Apical fragments taken from fronds with a diameter >2 mm were dragged through filtered seawater solidified with 0.8% agar and containing (μg mL^{-1}): penicillin G (300), streptomycin sulfate (100), neomycin (50), and kanamycin (200). They were then incubated for two days in the same antibiotic mixture in SWMD-1 medium (Chen and Taylor 1978), whereupon the procedure was repeated. To promote growth, the tissue was placed in medium containing kinetin ($10^{-6}M$) and α-napthaleneacetic acid (10^{-6} *M*) for one week. A segment was then excised near the apex and the cortical tissue was removed under aseptic conditions. Tissue cubes (2 mm on a side) were cut from unpigmented segments of subcortical and medullary tissue and were inoculated for four weeks in TC-1 medium (Chen and Taylor 1978) on a gyratory shaker. Those segments with the characteristic reddish-brown colour were found to be axenic after checking under oil immersion and SEM for bacteria.

Hypochlorite Treatments

Phaeophycophyta - Fries (1977) found that frond tips of *Fucus spiralis* and *Fucus vesiculosus* remained contaminated after antibiotic treatments and decided that a stronger sterilization method was needed. The tips of *F. spiralis* fronds were cut and immersed in a saturated calcium hypochlorite solution for 2-5 minutes and were then washed in sterile seawater prior to being placed on ASP6 F2 medium (Fries 1977) which had been solidified with 5 g agar per litre. Those pieces which appeared to be free of bacteria after one week were transferred to new plates and then to ASP6 F2 medium one week later. The segments had lost their colour except for a few cells in the apical pit region, some spots on the mid-rib or around the cryptostomata. Some cells began to divide within one month and differentiated into several shoots, while others remained dormant for several months. This method was not very efficient in producing axenic tissues for only four tips were determined to be bacteria-free out of "many hundred treated tips". Nevertheless, Fries (1984) was able to induce the growth of plantlets from the axenic culture of *F. spiralis.* Those tissues which were axenic grew much more slowly than plants supporting bacteria unless growth prom-

oting substances such as phenylacetic acid or p-hydroxyphenylacetic acid were added (Fries 1977).

Essentially the same procedure was used by Fries (1980) for sporophytes of *Laminaria digitata* and *Laminaria hyperborea,* except that pieces of tissue 5 x 5 mm from the thallus where the stipe broadens into a blade were dipped in saturated calcium hypochlorite for 30-60 s. Sterility was tested according to the methods of Fries (1970) but the success rate was not reported.

Potassium Tellurite

Potassium tellurite is a bacteriostatic agent which has been used in agar media to allow the algae to "outgrow" bacteria. Caution should be exercised in its use, as it is now labelled a teratogen.

Ducker and Willoughby (1964) washed apices of *Falkenbergia rufolanosa* in sterile seawater, dragged them through 1% agar containing 0.01% potassium tellurite and incubated them in light at 16°C. The alga was able to outgrow the bacteria in the agar and produce "clean" filaments; however, no axenicity test was conducted.

Rosowski and Hoshaw (1970) used a combination of techniques to isolate *Zygnema* sp. into axenic culture. Filaments were chopped with five razor blades spaced 1.5 mm apart and the resulting cells were placed in sterile distilled water and sonicated for 5 s. They were then centrifuged, washed and sonicated for an additional 5 s prior to being recentrifuged and washed 5 times in sterile distilled water. The suspension was then sprayed onto a 1.5% agar medium containing 10 mg L^{-1} potassium tellurite and incubated at room temperature. Bacteria-free filaments, that is those that did not give rise to bacterial colonies when streaked on nutrient agar, were then transferred to agar slants.

Detergents and Phenol

Contaminated cultures of *Chlorella pyrenoidosa, Anacystis nidulans* and *Chlamydomonas* sp. were apparently freed of bacteria by centrifuging the cells with a detergent and phenol. McDaniel *et al.* (1962) added 2 drops of laboratory detergent to a 50 mL culture containing 0.3-0.8 mL of centrifuge-packed cells and shook it rigorously, prior to mixing it with a 1% phenol solution for 20 s, allowing it to stand for 15 min and centrifuging for 5 min. The supernatant was discarded and distilled water added before centrifuging again for 5 min. The cells from the pellet were then plated out on Knop's medium with 0.5% glucose and 2% agar. The authors reported a 75% success rate, although the axenicity was only tested by examining gram-stained smears.

Antifungal Agents

Polyene Macrolide Antibiotics - Polyene macrolides contain a polyene chromophore in the lactone ring and show antifungal but no antibacterial behaviour (Dekker 1969). They are especially active against yeasts and filamentous fungi. Ghosh and Ghosh (1963a) found that the growth of *Candida albicans* was completely arrested by 1 μg mL^{-1} of amphotericin B and 5 μg mL^{-1} of nystatin. Similarly, the growth of *Neurospora crassa* was reduced 50% by concentrations of 0.054, 0.20 and 0.27 μg mL^{-1} of amphotericin B, filipin, and nystatin, respectively (Kinsky 1961). Divan and Schnoes (1982) used 5.0 μg mL^{-1} amphotericin B to rid a dinoflagellate culture of a filamentous fungus. Both Kinsky (1961) and Ghosh and Ghosh (1963b) observed that cytoplasmic constituents were lost following exposure to antibiotics. This latter finding led to the hypothesis that polyene antibiotics bind to sterols in the cell membrane causing the loss of selective permeability (Kinsky 1961, 1962; Dekker 1969).

Polyene antibiotics have a broad range of antifungal activity. Using an agar dilution method, Frank *et al.* (1958-59) showed that almost all phytopathogenic fungi tested

were sensitive to nystatin, with inhibitory concentrations ranging from ≤ 0.3 - 18 μg mL^{-1} agar over a 48-120 h period. However, some species within the Phycomycetes were resistant. *Phytophthora infestans* and *Pythium debaryanum* tolerated 35 and 1420 μg mL^{-1} agar, respectively. Similarly, Vaartaja (1960) reported that both *P. debaryanum* and *Pythopthora cactorum* were resistant to individual concentrations of 500 μg mL^{-1} of pimaricin, rimocidin and nystatin. Somewhat surprisingly, these antibiotics were not very effective against fungal plant diseases in the field. The reason is apparently related to their instability after application as a result of their sensitivity to UV light. For instance, the antifungal activity is lost within a few days in aqueous solution in daylight (Dekker 1969).

Algae and protozoa both contain sterols in the protoplast membranes and may be sensitive to polyene antibiotics. Lampen and Arnow (1961) exposed eight species of algae representing four divisions (Cyanochloronta, Chlorophycophyta, Euglenophycophyta, and Chrysophycophyta) to nystatin for 72 h and found that the growth of all species except *Navicula pelliculosa* was inhibited by concentrations ranging from 1 to 30 μg mL^{-1} (Table IV). Nystatin appeared to act by disrupting the ability of the algae to maintain elevated internal K^+ concentrations. The polyene macrolide antibiotics appear to be effective against most species of fungi and yeasts tested. Nevertheless, their selective toxicities towards algae should be evaluated before employing them as fungicides in algal cultures (see below).

Caffeine - Bowne (1964) reported that caffeine at concentrations of 0.01-0.03 *M* would kill fungi and non-photosynthetic protozoa and "if anything, stimulate the algae". *Euglena* and *Chlamydomonas* cultures were freed from species of *Aspergillus, Penicillium, Saccharomyces* and *Paramecium* by caffeine in various media including pond water, pea infusion, modified Cramer-Myers salt medium and Beijerinck's mineral medium with acetate.

Griseofulvin - The sensitivity of fungi to griseofulvin varies with the species tested (Brian 1949). For example, the growth of *Microsporum gypseum* and four species of *Trichophyton* was completely arrested at 10 μg mL^{-1} griseofulvin (El-Nakeeb et *al.* 1965). The growth of *Alternaria tenuis, Cercospora melonis* and *Glomerella cingulata* was inhibited 33-65% at 10 μg mL^{-1} while *Aspergillus niger, Fusarium nivale* and *Neurospora crassa* were only marginally affected (9-20% inhibition). Yeasts and bacteria were resistant to griseofulvin (Roth et *al.* 1959; Shockman and Lampen 1962; El-Nakeeb et *al.* 1965). Data on the effects of griseofulvin on algae are few. Mizukami

Table IV Sensitivity of algal growth to nystatin (adapted from Lampen and Arnow 1961).

Division	Organism	MIC[a] of nystatin (72 h) (μg mL^{-1})
Euglenophycophyta	*Euglena gracilis* no. 752	30
	E. gracilis no. 753	30
Chlorophycophyta	*Chlorella vulgaris*	3
	Scenedesmus obliquus	1
	Chlamydomonas reinhardti	1
	Prototheca zopfii	1
Chrysophycophyta	*Ochromonas malhamensis*	1
	Navicula pelliculosa	>60[b]
Cyanochloronta	*Plectonema boryanum*	1[b]

[a] Minimum inhibiting concentrations.
[b] Incubated for 5 days.

and Wada (1983) found that apical growth in *Bryopsis plumosa* exposed to 10 μg mL^{-1} griseofulvin for two days ceased. Densely packed protoplasm at the tip disappeared and new growth points were not induced. They suggested that the morphological abnormalities were the result of microtubule inhibition. Kiermayer and Meindl (1979)

Table V Concentrations of cycloheximide completely inhibiting the growth of species representative of the Cyanochloronta, Chlorophycophyta, Xanthophyceae, Bacillariophyceae, and Euglenophycophyta after a three-week exposure.

Group	*Species*	Cycloheximide (μg mL^{-1})
Cyanochloronta	*Microcystis aeruginosa*	NI[a,e]
		NI[b,f]
		NI[c,g]
	Anabaena cylindrica	NI[a,e]
		NI[d,e]
	Aphanocapsa sp.	NI[a,e]
	Gloeothece rupestris	NI[a,e]
	Coelosphaerium kuetzingianum	NI[a,e]
	Oscillatoria tenuis	NI[a,e]
	Lyngbya sp.	NI[a,e]
	Tolypothrix tenuis	NI[a,e]
	Tolypothrix sp.	NI[a,e]
	Gloeotrichia echinulata	NI[a,e]
	Phormidium foveolarum	NI[a,e]
	Phormidium sp.	NI[d,e]
	Cylindrospermum licheniforme	NI[c,g]
Chlorophycophyta	*Ankistrodesmus falcatus*	1[a]
	Haematococcus lacustris	1[a]
		1[d]
	Scenedesmus obliquus	2[c]
		1[a]
	Raphidonema longiseta	1[a]
	Hormidium subtile	20[a]
	Chlorella variegata	NI[c,g]
	Chlorella pyrenoidosa	20[a]
	Chlamydomonas agloeformis	50[a]
		50[d]
Chrysophycophyta		
Xanthophyceae	*Tribonema aequale*	1[a]
	Polydriella helvetica	50[d]
Bacillariophyceae	*Navicula minima*	20[a]
	Navicula pelliculosa	2[d]
	Gomphonema parvulum	2[c]
	Nitzschia palea	2[c]
Euglenophycophyta	*Euglena gracilis*	100[d]

[a] Zehnder and Hughes (1958).
[b] Vance (1966)-length of exposure of *M. aeruginosa* not given.
[c] Palmer and Maloney (1955).
[d] Hunter and McVeigh (1961).
[e] Not inhibited by the maximum concentration tested (200 μg mL^{-1}).
[f] Not inhibited by the maximum concentration tested (1 μg mL^{-1}).
[g] Not inhibited by the maximum concentration tested (2 μg mL^{-1}).

did observe microtubule inhibition in *Micrasterias denticulata* exposed to 0.01-0.001% griseofulvin. Because griseofulvin is effective against a limited number of fungi and may be toxic to algae, it cannot be regarded as a general fungicide for cleaning algal cultures.

Selectivity Toxic Agents Allowing Differentiation Between Algae

Agents Used to Purify Cyanochloronta Cultures - Cycloheximide - Representative species of Cyanochloronta, Chlorophycophyta, Chrysophycophyta and Euglenophycophyta have been subjected singly to cycloheximide concentrations ranging from 1 to 1000 μg mL^{-1} over a 3-week period to determine whether selective toxicity existed (Table V). In general, the blue-green algae were unaffected by cycloheximide whereas members of the other groups had their growth rates inhibited at $\leq$ 100 μg mL^{-1}. The green together with the yellow-green alga and diatoms suffered arrested growth at $\leq$ 50 μg mL^{-1}. Berland and Maestrini (1969b) also found cycloheximide to be toxic to diatoms as five species exposed to 5 μg mL^{-1} died. *Euglena gracilis* was the only species where 100 μg mL^{-1} was necessary to inhibit growth. The growth of *Chlorella variegata* was not affected by 2 μg mL^{-1} cycloheximide, the only concentration which was tested, but *C. pyrenoidosa* was inhibited at 20 μg mL^{-1}.

Zehnder and Hughes (1958) observed the growth of *Gloeothece rupestris* over 5 weeks (Table V) and reported that at 100 and 200 μg mL^{-1} cycloheximide growth was "less vigorous" than the controls (50 μg mL^{-1} was ineffective). In another experiment, a mixed culture of eight species of blue-green and nine species of green algae was grown in cycloheximide at levels from 0-100 μg mL^{-1}. After 27 days at the highest concentration, only blue-green algae were present. Zehnder and Hughes (1958) then took samples of algae from the natural environment (e.g. waterbloom, moist soil, wet

Table VI Concentrations of nystatin completely inhibiting the growth of species representative of Cyanochloronta, Chlorophycophyta, Xanthophyceae, Bacillariophyceae, and Euglenophycophyta after a three-week exposure (adapted from Hunted and McVeigh 1961).

Group	*Species*	Nystatin (μg mL^{-1})
Cyanochloronta	*Anabaena cylindrica*	NI[a]
	Fremiyella diplosiphon	NI
	Nostoc sp.	NI
	Phormidium sp.	200
Chlorophycophyta	*Hormidium* sp.	1
	Chlorococcum minutum	1
	Ankistrodesmus falcatus	20
	Chlamydomonas agloeoformis	20
	Chlorella pyrenoidosa	50
	Stichococcus bacillaris	100
	Haematococcus lacustris	200
	Scenedesmus obliquus	NI[a]
	Goccomyxa elongata	NI
Chrysophycophyta Xanthophyceae	*Polydriella helvetica*	200
Bacillariophyceae	*Navicula pelliculosa*	100
Euglenophycophyta	*Euglena gracilis*	50

[a] No inhibition at highest concentration tested (200 μg mL^{-1}).

moss), and discovered that 50 μg mL^{-1} cycloheximide suppressed all of the green algae and diatoms and allowed the cyanophytes to flourish. Hunter and McVeigh (1961) took algae which had been completely inhibited by cycloheximide, but which still had apparently viable cells, and cultured them for one week after the test. No growth occurred. Therefore cycloheximide can be useful in isolating cyanobacterial cultures from algal contaminants and fungi, but not from bacteria (see above).

Nystatin - Hunter and McVeigh (1961) exposed representatives of the Cyanochloronta, Chlorophycophyta, Chrysophycophyta and Euglenophycophyta to concentrations of nystatin ranging from 1-200 μg mL^{-1} (Table VI) and found that, in general, blue-green algae were tolerant while others were not. Of the four species of Cyanochloronta tested, only *Phormidium* sp. was inhibited at 200 μg mL^{-1}. The growth of the remaining species in Table VI was completely arrested at $\leq$ 200 μg mL^{-1}, except in the cases of *Scenedesmus obliquus* and *Goccomyxa elongata*.

It would appear from a comparison of Tables V and VI that nystatin is not as effective as cycloheximide. However, in most cases its mycocidal effect would be useful to decrease the fungal contamination of blue-green algae.

Agents used to eliminate Cyanochloronta - Blue-green algae are more sensitive to streptomycin than green algae or diatoms (Foter *et al.* 1953; Palmer and Maloney 1955; Jacob and Talpasayi 1977; Harrass *et al.* 1985). Table VII shows that the minimum inhibitory concentration (MIC) for blue-greens lies in the range 0.09-2.0 μg mL^{-1}. Considering that Palmer and Maloney (1955) used only a single concentration of 2.0 μg mL^{-1} to test different algal species including *Microcystis aeruginosa,* and that Harrass et al. (1985) determined an MIC for *M. aeruginosa* of only 0.28 μg mL^{-1} (Table VII), it is likely that the range is actually smaller (0.09-0.86 μg mL^{-1}), indicating a relatively uniform sensitivity to streptomycin.

With the exception of *Chlamydomonas reinhardtii* (MIC = 0.66 μg mL^{-1}), all of the green algae tested were at least 2-3 times more resistant to streptomycin than the blue-greens, although the range of sensitivity was greater (2.0-66.0 μg mL^{-1}), indicating that no single species would be representative of the group. Similarly, the diatoms also displayed a greater, but more uniform tolerance, with MIC's from 2 to 9 μg mL^{-1} (Table VII).

The data from the different sources in Table VII agree well, with the exception of those for *Scenedesmus obliquus*. Harrass *et al.* (1985) reported an MIC of 21.0 μg mL^{-1} whereas Palmer and Maloney (1955) observed a complete inhibition of growth at 2.0 μg mL^{-1}. This difference remains unexplained. To ascertain whether streptomycin was selectively toxic, Harrass et al. (1985) set up a paired culture containing *Anabaena cylindrica* (MIC = 0.28 μg mL^{-1}) and *S. obliquus* (MIC = 21.0 μg mL^{-1}) in media containing 6.6 μg mL^{-1} streptomycin. Within 4 days growth of *A. cylindrica* had stopped, leading to a unialgal culture of *S. obliquus*.

Bacitracin was also more toxic to blue-green algae than to other algae (Hunter and McVeigh 1961). *A. cylindrica* and *Phormidium* sp. were completely inhibited by 2 μg mL^{-1} bacitracin while 50 μg mL^{-1} was necessary to arrest the growth of *Navicula pelliculosa*. Several species: *Polydriella helvetica, H. lacustris, C. agloeoformis* and *E. gracilis,* were resistant to the highest concentration tested, 200 μg mL^{-1}.

Blue-green algae have also been reported to be sensitive to neomycin (Foter et al. 1953; Jacob and Talpasayi 1977), although whether neomycin is selectively toxic is an open question. From the limited information available, blue-green algae showed the same sensitivity (6 μg mL^{-1}) as *Chlamydomonas* sp., *Gomphonema* sp., and *Nitzschia* sp. *Scenedesmus* exhibited a greater resistance as it was inhibited at 25-49 μg mL^{-1} (Foter et al. 1953). Jacob and Talpasayi (1977) provided no data, but mentioned that erythromycin was equally inhibitory, but not lethal, to 14 blue-green algae tested, and that penicillin had little effect.

Table VII Minimum inhibitory concentrations (MIC) of streptomycin that arrested the growth of representative species of Cyanochloronta, Chlorophycophyta and Chrysophycophyta.

Group	Species	MIC ($\mu g\ mL^{-1}$)	Exposure Time (days)
Cyanochloronta	*Microcystis aeruginosa*	2.0[a,d]	21
	Microcystis aeruginosa	0.28[b]	35
	Lyngbya sp.	0.09[b]	35
	Oscillatoria tenuis	0.28[b]	35
	Anabaena cylindrica	0.28[b]	35
	Anabaena flos-aquae	0.28[b]	35
	Aphanizomenon flos-aquae	0.86[b]	35
	Nostoc sp.	<2.0[c]	30
	Phormidium sp.	<2.0[c]	30
	Cylindrospermum licheniforme	2.0[a]	21
Chlorophycophyta	*Chlamydomonas reinhardtii*	0.66[b]	35
	Chlamydomonas sp.	9-18[c]	30
	Pediastrum sp.	2.1[b]	35
	Selenastrum capricornutum	2.1[b]	35
	Ankistrodesmus sp.	6.6[b]	35
	Stigeoclonium sp.	6.6[b]	35
	Scenedesmus obliquus	2.0[a]	21
	Scenedesmus obliquus	21.0[b]	35
	Scenedesmus sp.	18-36[c]	30
	Ulothrix sp.	21.0[b]	35
	Chlorella vulgaris	66.0[b]	35
	Chlorella variegata	NI[a,e]	21
Chrysophycophyta	*Navicula* sp.	6.6[b]	35
	Gomphonema parvulum	NI[a,e]	21
	Gomphonema sp.	4-9[c]	30
	Nitzschia sp.	2-4[c]	30
	Nitzschia palea	2.0[a]	21

[a] Palmer and Maloney (1955).
[b] Harrass et al. (1985).
[c] Foter et al. (1953)
[d] 2.0 $\mu g\ mL^{-1}$ only concentration tested.
[e] Not inhibited by 2.0 $\mu g\ mL^{-1}$.

It appears that streptomycin and bacitracin would be the antibiotics of choice to eliminate blue-green contaminants from other algal cultures.

Agents Used to Eliminate Bacilliarophyceae

Germanium Dioxide - Lewin (1966) examined the selective toxicity of germanium compounds against a mixed culture containing blue-green, green, brown, and red algae. These were inoculated into artificial seawater with concentrations of germanium dioxide (GeO_2) ranging from 0-100 mg L^{-1}. After one week the suspensions were streaked onto agar plates. No diatoms were found from cultures containing $\geq$3.0 mg L^{-1} GeO_2. In a further experiment with pure cultures of diatoms (2 species of *Amphipora* and *Nitzschia*, 4 strains of *Cylindrotheca closterium*, 2 strains of *Phaeodactylum tricornutum, Navicula incerta, Skeletonema costatum, Cyclotella cryptica*) exposed to 1, 3, and 10 mg L^{-1} GeO_2 for 12 days, the average growth rate was inhibited 42, 67, and 83%, respectively. Lewin (1966) postulated that GeO_2 could take part in reactions similar to those of SiO_2, with germanium replacing silicon in the final

mineralized shell. She recommended concentrations of 1-10 mg L^{-1} GeO_2 to rid algal cultures of diatoms.

Borden and Stein (1969) used 6-10 mg L^{-1} GeO_2 to control diatoms in cultures of the green alga, *Codium fragile*. Similarly, Chen *et al.* (1969; 1970) used GeO_2 to eliminate diatoms from cultures of red algae. However, problems with selective toxicity of GeO_2 towards brown algae emerged. McLachlan *et al.* (1971) noted that with 4 species of *Fucus*, an exposure of several days to 5-10 mg L^{-1} GeO_2 resulted in apical necrosis of embryos and mature thalli even though the diatoms were not suppressed. Tatewaki and Mizuno (1979) reported that 2.5-5.0 mg L^{-1} GeO_2 inhibited the growth of all brown algae examined but had no effect on that of the green and red algae tested.

Markham and Hagmeier (1982), after observing that a *Laminaria saccharina* culture stopped growing suddenly after a routine addition of GeO_2 to control diatoms, proceeded to test diatoms and macroalgae for their susceptibility to GeO_2. Diatoms were subjected to 0.009-1.79 mg L^{-1} GeO_2 for 4-9 days at 10 and 18°C. *Chaetoceros didymus* and *Rhizosolenia delicatula* were strongly inhibited by 0.045 and 0.134 mg L^{-1} GeO_2, respectively. *Thalassiosira rotula* displayed a 50% growth inhibition at 0.18 mg L^{-1} GeO_2. *Coscinodiscus concinnus* was unaffected. The effects on growth were independent of temperature in the range studied.

In a 6-day test, GeO_2 at concentrations ranging from 0.22-8.95 mg L^{-1} had no adverse effects on the red algae: *Polysiphonia urceolata, Porphyra umbilicalis,* and *Chondrus crispus*. The only green species tested, *Ulva lactuca,* exhibited a decline in % diameter increase d^{-1} (7.4% compared with 9.0% for the controls) at the maximum concentration used. With regard to brown algae, the specific growth rate (% length increase d^{-1}) of *Fucus spiralis* decreased from 4.1 to 3.2% at 8.95 mg L^{-1} GeO_2, but the most significant effect was observed for *Laminaria saccharina* where GeO_2 at 3.5 and 8.95 mg L^{-1} resulted in a growth inhibition of 50 and 67%, respectively. Although the latter species appeared healthy, the fronds were brittle, as they broke into pieces when handled. Germanium is reported to interfere with silicon metabolism, blocking the initial uptake of silicate and inhibiting the metabolism of that already accumulated, thereby preventing cell division by arresting wall formation (Markham and Hagmeier 1982). Parker (1969) noted that silicon was present in some brown algae, thus the lack of silicon may have led to the observed brittleness of the *Laminaria* thallus. Darley and Volcani (1969) reported that the molar ratio of Ge to Si is important in the mode of action of GeO_2. Using the test organism *Cylindrotheca fusiformis,* they showed that wall formation was inhibited at Ge/Si ratios of 0.1 and 0.2. However, at a Ge/Si ratio of 0.5, DNA synthesis and growth of the diatom were inhibited. Markham and Hagmeier (1982) suggested that lower levels of GeO_2 be used with brown algae, in the range of 0.045-0.179 mg L^{-1} as this appears to be "safe" for them, while still eliminating most diatoms.

Algal Metabolites Affecting Other Algae and Microrganisms

Many algae excrete substances which affect other algal species as well as bacteria and fungi. Juttner (1979) found that an excretion product of *Cyanidium caldarium,* geranylacetone, at a concentration of 20 mg mL^{-1} almost totally inhibited the carotenoid synthesis of *Synechococcus* 6911. Jorgensen (1956) reported that algal metabolites from one species could influence the growth of another. For example, *Chlorella pyrenoidosa* cultured separately in 21-day filtrates of *Scenedesmus quadricauda* and *Nitzschia palea* exhibited an inhibition of growth with the former and an acceleration of growth in the latter. *S. quadricauda* grew more quickly in 21-day filtrates of both *C. pyrenoidosa* and *N. palea.*

Some of the antimicrobial and antifungal algal metabolites are listed in Table VIII. Unfortunately, most of the test organisms employed to ascertain their activity are not

Table VIII The antimicrobial effects of algal metabolites.

Group	*Species*	Metabolite	Organism Inhibited
Cyanochloronta	*Lyngbya majuscula*	δ-lactone	*Mycobacterium smegmatis*[a]
		δ-lactone	*Streptococcus pyogenes*[a]
		-	*Candida albicans*[b]
		-	*Penicillium* sp.[b]
		-	*Rhizopus oryzae*[b]
		-	*Cryptococcus neoformans*[b]
Chlorophycophyta	*Caulerpa racemosa*	-	*Candida albicans*[b]
			Penicillium sp.[b]
			Rhizopus oryzae[b]
			Cryptococcus neoformans[b]
	Halimeda sp.	trialdehyde	marine bacteria & fungi[a]
		halimedatrial	marine bacteria & fungi[a]
Phaeophycophyta	*Dictyota indica*	-	*Candida albicans*[b]
			Penicillium sp.[b]
			Rhizopus oryzae[b]
			Cryptococcus neoformans[b]
	Dictyopteris zonaroides	zonarol, isozonarol	*Phytophthora cinnamomi*[b]
			Rhizoctonia solani[c]
			Sclerotinia scleratiorum[c]
			Sclerotium rolfii[c]
	Dictyopteris undulata	farnesylhydroquinone	antimicrobial[a]
		yakuzanol	
	Sargassum kjellmanianum	kjellmanianone	antibacterial[a]

Rhodophycophyta	*Laurencia obtusa*	-	*Candida albicans*[b]
			Penicillium sp.[b]
			Rhizopus oryzae[b]
			Cryptococcus neoformans[b]
	Laurencia yamada	chondriol	antiviral & antimicrobial[c]
	Laurencia brongniaratii	brominated indoles	antimicrobial[a]
	Laurencia sp.	laurinterol	*Staphlococcus aureus*[c]
		debromolaurinterol	*Mycobacterium smegmatis*[c]
			Candida albicans[c]
	Chondria oppositiclada	cycloeudesmol	*Staphlococcus aureus*[c]
			Mycobacterium smegmatis[c]
			Candida albicans[c]
	Chondria californica	sulphone	*Vibrio anguilarum*[c]
			Proteus mirabilis[c]
			Salmonella typhimurium[c]
			Escherichia coli[c]

[a] Faulkner 1984.
[b] Accorinti 1983.
[c] Faulkner 1978.

normally found as algal epiphytes. The blue-green alga, *Lyngbya majuscula*, produces substances effective against both bacteria (*Mycobacterium smegmatis, Streptococcus pyogenes*) and fungi (*Candida albicans, Penicillium* sp., *Rhizopus oryzae*, and *Cryptococcus neoformans*). It also secretes inflammatory toxins lyngbyatoxin A and debromoaplysiatoxin which cause contact dermatitis known as "swimmers itch" (Moore 1981).

The antifungal metabolites are widespread as they are found in green (*Caulerpa racemosa, Halimeda* sp.), brown (*Dictyota indica, Dictyopteris zonaroides*) and red algae (*Laurencia* sp., *L. obtusa, Chondria oppositiclada)* (Table VIII). Accorinti (1983) speculated that the inhibitory effects on fungi could be due to polyene antibiotics (see above). The antimicrobial activity of many marine algae appears to be due to the production of brominated phenols and sesquiterpene phenols (Faulkner 1978). For example, laurinterol and debromolaurinterol isolated from several species of *Laurencia* and cycloeudesmol from *Chondria oppositiclada* inhibited *Staphlococcus aureus, M. smegmatis* and *C. albicans* (Table VIII). Sulfur-containing compounds such as sulphone from *Chondria oppositiclada* have also shown antimicrobial activity (Table VIII). These are some of the algal metabolites exhibiting antimicrobial and antifungal behaviour. As more natural products are isolated, it is probable that more compounds active against bacteria and fungi will be discovered.

The techniques reviewed here to purify algal cultures show that there is no single method applicable for every species. It does not appear to date that antimicrobial and antifungal products from the algae themselves have been used to rid cultures of epiphytic contamination. Different algae will in all probability be contaminated with a wide spectrum of bacteria and, to a lesser extent, fungi and other algae, each of which will differ in sensitivity to the various chemical agents used to eliminate them. A mixture of chemicals, rather than a single agent, has been necessary to prepare axenic cultures. In addition, closely related species of algae may exhibit quite different responses to the same chemical. It will therefore be necessary to experiment with different types and concentrations of chemicals, keeping in mind that they must be selectively toxic. The techniques gathered here provide the reader with background information and may suggest useful approaches for the isolation of algae into pure culture.

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References

Accortini, J. 1983. Antifungal product from algal origin (review). *Rev. Int. Ocean. Med.* 72: 45-53.

Antia, N.J. and **Kalmakoff, J.** 1965. Growth rates and cell yields from axenic mass culture of fourteen species of marine phytoplankters. *Fish. Res. Bd. Can. Manus. Rep. Ser.* 203. 24 pp.

Benson, H.J. 1985. *Microbial applications.* A laboratory manual in general microbiology. W.C. Brown, Dubuque, Iowa. 450 pp.

Berglund, H. 1969. On the cultivation of multicellular marine green algae in axenic culture. *Sv. Bot. Tidskr.* 63: 251-264.

Berland, B.R. and **Maestrini, S.Y.** 1969a. Study of bacteria associated with marine algae in culture. II. Action of antibiotic substances. *Mar. Biol.* 3: 334-335.

Berland, B.R. and **Maestrini, S.Y.** 1969b. Action de quelques antibiotiques sur le développement de cinq diatomées en culture. *J. Exp. Mar. Biol. Ecol.* 3: 62-75.

Berland, B.R., Bianchi, M.G.and **Maestrini, S.Y.** 1969. *Etude des bactéries associées aux algues marines en culture. I. Détermination préliminaire des espèces. Mar. Biol.* 2: 350-355.

Bold, H.C. and **Wynne, M.J.** 1978. Introduction to the algae. Structure and reproduction. Prentice-Hall, Inc. Englewood Cliffs, N.J. 706 pp.

Bordon, C.A.and **Stein, J.R.** 1969. Reproduction and early development in *Codium fragile* (Suringar) Hariot: Chlorophyceae. Phycologia 8: 91-99.

Bowne, S.W. Jr. 1964. Purification of algal cultures with caffeine. *Nature* 204: 801.

Bradley, P.M., Cheney, D.P. and **Saga, N.** 1988. One step antibiotic disc method for obtaining axenic cultures of multicellular marine algae. *Plant Cell Tissue Organ Cult.* 12: 55-60.

Brian, P.W. 1949. Studies on the biological activity of griseofulvin. *Ann. Bot.,* Lond. 13: 59-77.

Brown, L.M. 1982. Production of axenic cultures of algae by an osmotic method. *Phycologia* 21: 408-410.

Brown, R.M., Jr. and **Bischoff, H.W.** 1962. A new and useful method for obtaining axenic cultures of algae. *Phycol. News Bull.* 15: 43-44.

Chen, L.C.-M., Edelstein, T. and **McLachlan, J.** 1969. *Bonnemaisonia hamifera* Hariot in nature and in culture. *J. Phycol.* 5: 211-220.

Chen, L.C.-M., Edelstein, T., Ogata, E. and **McLachlan, J.** 1970. The life history of *Porphyra miniata. Can. J. Bot.* 48: 385-389.

Chen, L.C.-M. and **Taylor, A.R.A.** 1978. Medullary tissue culture of the red alga *Chondrus crispus. Can. J. Bot.* 56: 883-886.

Cheney, D.P., Mar, E., Saga, N. and **van der Meer, J.** 1986. Protoplast isolation and cell division in the agar producing seaweed *Gracilaria* (Rhodophyta). *J. Phycol.* 22: 238-243.

CRC 1980a. Handbook of antibiotic compounds. Vol. I. Carboydrate antibiotics. Bérdy, J., Aszalos, A., Bostian, M. and McNitt, K.L. CRC Press, Inc. Boca Raton, Florida. 451 pp.

CRC 1980b. Handbook of antibiotic compounds. Vol. II. Macrocyclic lactone (lactam) antibiotics. Bérdy, J. Aszalos, A., Bostian, M. and McNitt, K.L. CRC Press, Inc., Boca Raton, Florida. 534 pp.

CRC 1980c. Handbook of antibiotic compounds. Vol. III. Quinone and similar antibiotics. Bérdy, J., Aszalos, A., Bostian, M. and McNitt, K.L. CRC Press, Inc., Boca Raton, Florida, 422 pp.

CRC 1980d. Handbook of antibiotic compounds. Vol. IV. Amino acid, peptide antibiotics. Bérdy, J., Aszalos, A., Bostian, M. and McNitt, K.L. CRC Press, Inc., Boca Raton, Florida, 558 pp.

CRC 1980e. Handbook of antibiotic compounds. Vol. VI. Alicyclic and aromatic antibiotics. Bérdy, J., Aszalos, A., Bostian, M. and McNitt, K.L. CRC Press, Inc., Boca Raton, Florida, 460 pp.

Darley, W.M. and **Volcani, B.E.** 1969. Role of silicon in diatom metabolism. *Exp. Cell Res.* 58: 334-342.

Dekker, J. 1969. Antibiotics. *In* Fungicides Vol. II. Chemistry and Physiology. *Edited by* D.C. Torgeson. Academic Press, New York. pp. 580-635.

Divan, C.L. and **Schnoes, H.K.** 1982. Production of axenic *Gonyaulax* cultures by treatment with antibiotics. *Appl. Environ. Microbiol.* 44: 250-254.

Droop, M.R. 1967. A procedure for routine purification of algal cultures with antibiotics. *Br. Phycol. Bull.* 3: 295-297.

Druehl, L.D. and **Hsiao, S.I.C.** 1969. Axenic culture of Laminariales in defined media. *Phycologia* 8: 47-49.

Ducker, S.C. and **Willoughby, L.G.** 1964. Potassium tellurite as a bacteriostatic agent in isolating algae. *Nature* 202: 210.

El-Nakeeb, M.A., McLellan, W.L., Jr., and **Lampen, J.O.** 1965. Antibiotic action of griseofulvin on dermatophytes. *J. Bacteriol.* 89: 557-563.

Ebringer, L. 1964. Bleaching of euglenas by antibiotics - a specific form of antagonism in actinomycetes. *Folia Microbiol.* 9: 249-255.

Faulkner, D.J. 1978. Antibiotics from marine organisms. *In* Topics in Antibiotic Chemistry. Vol. 2. *Edited by* P. Sammes. Ellis Horwood. pp. 9-58.

Faulkner, D.J. 1984. Marine natural products: metabolites of marine algae and herbivorous marine molluscs. *Natural Prod. Rep.* 1: 251-280.

Foter, M.J., Palmer, C.M. and **Maloney, T.E.** 1953. Antifungal properties of various antibiotics. Antibiot. Chemother. 3: 505-508.

Frank, E., Pansy, F.E. and **Pagano, J.F.** 1958-1959. Nystatin in the control of fungal infections of orchids. Antibiot. Annu. pp. 898-902.

Fries, L. 1963. On the cultivation of axenic red algae. *Physiol. Plant.* 16: 695-708.

Fries, L. 1970. The influence of microamounts of organic substances other than vitamins on growth of some red algae in axenic culture. *Br. Phycol. J.* 5: 39-46.

Fries, L. 1977. Growth regulating effects of phenylacetic acid and p-hydroxyphenylacetic acid on *Fucus spiralis* L. (Phaeophyceae, Fucales) in axenic culture. *Phycologia* 26: 451-455.

Fries, L. 1980. Axenic tissue cultures from the sporophytes of *Laminaria digitata* and *Laminaria hyperborea* (Phaeophyta). *J. Phycol.* 16: 475-477.

Fries, L. 1984. Induction of plantlets in axenically cultivated rhizoids of *Fucus spiralis*. *Can. J. Bot.* 62: 1616-1620.

Galloway, R.A. and **Krauss, R.W.** 1959. The differential action of chemical agents, especially polymyxin-B, on certain algae, bacteria and fungi. *Am. J. Bot.* 46: 40-49.

Gibor, A. and **Izawa, M.** 1963. The DNA content of the chloroplasts of *Acetabularia*. *Proc. Nat. Acad. Sci.* U.S.A. 50: 1164-1169.

Gibor, A., Polne, M., Biniaminov, M. and **Neushul, M.** 1981. Exploratory studies of vegetative propagation of marine algae: Procedure for obtaining axenic tissues. *Proc. Int. Seaweed Symp.* 10: 587-593.

Ghosh, A. and **Ghosh, J.J.** 1963a. Effect of nystatin and amphotericin B on the growth of *Candida albicans*. *Ann. Biochem. Exp. Med.* 23: 29-44.

Ghosh, A. and **Ghosh, J.J.** 1963b. Release of intracellular constituents of *Candida albicans* in presence of polyene antibiotics. *Ann. Biochem. Exp. Med.* 23: 611-626.

Green, B., Heilporn, V., Limbosch, S., Bolonkhere, M. and **Brachet, J.** 1967. The cytoplasmic DNAs of *Acetabularia mediterranea*. *Proc. Nat. Acad. Sci.* U.S.A. 58: 1351-1358.

Guillard, R.R.L. and **Ryther, J.H.** 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Dentonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 8: 229-239.

Harrass, M.C., Kindig, A.C. and **Taub, F.B.** 1985. Responses of blue-green and green algae to streptomycin in unialgal and paired culture. *Aquat. Toxicol.* 6: 1-11.

Hunter, E.O., Jr. and **McVeigh, I.** 1961. The effects of selected antibiotics on pure cultures of algae. *Am. J. Bot.* 48: 179-185.

Iwasaki, H. 1965. Nutritional studies of the edible seaweed *Porphyra tenera* I. The influence of different B_{12} analogues, plant hormones, purines and pyrimidines on the growth of conchocelis. *Plant Cell Physiol.* 6: 325-336.

Jacob, L. and **Talpasayi, E.R.S.** 1977. Antibiotic sensitivity of certain blue-green algae. *Acta. Bot. Indica* 5: 23.

Jones, A.K., Rhodes, M.E. and **Evans, S.C.** 1973. The use of antibiotics to obtain axenic cultures of algae. *Br. Phycol. J.* 8: 185-196.

Jorgensen, E.G. 1956. Growth inhibiting substances formed by algae. *Physiol. Plant.* 9: 712-726.

Juttner, F. 1979. The algal excretion product, geranylacetone: A potent inhibitor of carotene biosynthesis in *Synechoccus. Z. Naturforsch.* 34c: 957-960.

Kiermayer, O., and **Meindl, U.** 1979. Anti-microtubule action of various substances in *Micrasterias denticulata. Europ. J. Cell Biol.* 19: 130.

Kinsky, S.C. 1961. Alterations in the permeability of *Neurospora crassa* due to polyene antibiotics. *J. Bacteriol.* 82: 889-897.

Kinsky, S.C. 1962. Effect of polyene antibiotics on protoplasts of *Neurospora crassa. J. Bacteriol.* 83: 351-358.

Lampen, J.O. and **Arnow, P.** 1961. Inhibition of algae by nystatin. *J. Bacteriol.* 82: 247-251.

Lewin, J. 1966. Silicon metabolism in diatoms. V. Germanium dioxide, a specific inhibitor of diatom growth. *Phycologia* 6: 1-12.

Machlis, L. 1962. The nutrition of certain species of the green alga *Oedogonium. Am. J. Bot.* 49: 171-177.

Markham, J.W. and **Hagmeier, E.** 1982. Observations on the effects of germanium dioxide on the growth of macro-algae and diatoms. *Phycologia* 21: 125-130.

McDaniel, H.R., Middlebrook, J.B. and **Bowman, R.O.** 1962. Isolation of pure cultures of algae from contaminated cultures. *Appl. Microbiol.* 10: 223.

McLachlan, J., Chen, L.C.-M. and **Edelstein, T.** 1971. The culture of four species of *Fucus* under laboratory conditions. *Can. J. Bot.* 49: 1463-1469.

Millner, P.A., Callow, M.E. and **Evans, L.V.** 1979. Preparation of protoplasts from the green alga *Enteromorpha intestinalis* (L.) Link. *Planta* 147: 174-177.

Mizukami, M. and **Wada, S.** 1983. Morphological anomalies induced by antimicrotubule agents in *Bryopsis plumosa. Protoplasma* 114: 151-162.

Moore, R.E. 1981. Toxins from marine blue-green algae. *Environ. Sci. Res.* 20: 15-21.

Palmer, C.M. and **Maloney, T.E.** 1955. Preliminary screening for potential algicides. *Ohio J. Sci.* 55: 1-8.

Pappas, G.D. and **Hoffman, H.** 1952. The use of antibiotics for obtaining bacteria-free cultures of euglena. *Ohio J. Sci.* 52: 102-105.

Parker, B.C. 1969. Occurence of silica in brown and green algae. *Can. J. Bot.* 47: 537-540.

Pinter, I.J. and **Provasoli, L.** 1968. Heterotrophy in subdued light of 3 *Chrysochromulina* species. *Bull. Misaki Mar. Biol. Inst.* 12: 25-31.

Polne, M., Gibor, A. and **Neushul, M.** 1980. The use of ultrasound for the removal of macro-algal epiphytes. *Bot. Mar.* 23: 731-734.

Polne-Fuller, M. and **Gibor, A.** 1984. Developmental studies in *Porphyra.* I. Blade differentiation in *Porphyra perforata* as expressed by morphology, enzymatic digestions and protoplast regeneration. *J. Phycol.* 20: 609-616.

Provasoli, L. 1958. Effect of plant hormones on *Ulva. Biol. Bull.* 114: 375-384.

Provasoli, L. 1963. Growing marine seaweeds. *Proc. Int. Seaweed Symp.* 4: 9-17.

Provasoli, L. 1966. Media and prospects for the cultivation of marine algae. *In* Cultures and collections of algae. *Edited by* A. Watanabe and A. Hattori. Proc. U.S.-Japan Conf. Hakone, Japan. Sept. 12-15, 1966. pp. 63-75.

Provasoli, L., Pintner, I. and **Packer, L.** 1951. Use of antibiotics in obtaining pure cultures of algae and protozoa. *Proc. Am. Soc. Protozool.* 2: 6.

Provasoli, L., McLaughlin, J.J.A. and **Droop, M.R.** 1957. The development of artificial media for marine algae. *Arch. Mikrobiol.* 25: 392-428.

Rosowski, J.R. and **Hoshaw, R.W.** 1970. Cultivation of a filamentous alga in quantity on agar plates. *J. Phycol.* 6: 219-221.

Roth, F.J., Jr., Sallman, B. and **Blank, H.** 1959. *In vitro* studies of the antifungal antibiotic griseofulvin. *J. Invest. Dermat.* 33: 403-418.

Saga, N. and **Sakai, Y.** 1982. A new method for pure culture of macroscopic algae, the one step selection method. *Jpn. J. Phycol.* 30: 40-43.

Saga, N. and **Sakai, Y.** 1983. Axenic tissue culture and callus formation of the marine brown alga *Laminaria angustata*. *Bull. Jpn. Soc. Sci. Fish.* 49: 1561-1563.

Sheath, R.G. 1975. Formation of floridean starch: effects of various antibiotics. *J. Phycol.* 11: Suppl. p. 11.

Shockman, G.D. and **Lampen, J.O.** 1962. Inhibition by antibiotics of the growth of bacterial and yeast protoplasts. *J. Bacteriol.* 84: 508-512.

Soli, G. 1964. A system for isolating phytoplankton organisms in unialgal and bacteria-free culture. *Limnol. Oceanogr.* 9: 265-268.

Speck, R.S., Jarwetz, E. and **Gunnison, J.B.** 1951. Studies on antibiotic synergism and antagonism. *Arch. Intern. Med.* 88: 168-174.

Spencer, C.P., 1952. On the uses of antibiotics for isolating bacteria-free cultures of marine phytoplankton organisms. *J. Mar. Biol. Assoc. U.K.* 31: 97-106.

Stein, J.R. 1973. Editor. Handbook of phycological methods. Culture methods and growth measurements. Cambridge University Press. 448 pp.

Tatewaki, M. and **Provasoli, L.** 1964. Vitamin requirements of three species of *Antithamnion*. *Bot. Mar.* 6: 193-203.

Tatewaki, M. and **Mizuno, M.** 1979. Growth inhibition by germanium dioxide in various algae, especially in brown algae. *Jpn. J. Phycol.* 27: 205-212.

Tchan, Y.T. and **Gould, J.** 1961. Use of antibiotics to purify blue-green algae. *Nature* 192: 1276.

Tipper, D.J., and **Wright, A.** 1979. The structure and biosynthesis of bacterial cell walls. *In* The bacteria. Vol. 7. Mechanisms of adaptation. *Eds.* J.R. Sokatch and L.N. Ornston. Academic Press, New York. pp. 291-426.

Vaartaja, O. 1960. Selectivity of fungicidal materials in agar cultures. *Phytopathology* 50: 870-873.

Vance, B.D. 1966. Sensitivity of *Microcystis aeruginosa* and other blue-green algae and associated bacteria to selected antibiotics. *J. Phycol.* 2: 125-128.

Zehnder, A. and **Hughes, E.O.** 1958. The antialgal activity of acti-dione. *Can. J. Microbiol.* 4: 399-408.

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VENTILATION OF A TUNNEL BENEATH NORTHUMBERLAND STRAIT

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After brief comparison of ventilation schemes used in three long European tunnels, several possible methods of ventilating a 13 km submarine vehicular tunnel are examined. Probably the most suitable is one supplying fresh air from a smaller ventilation tunnel parallel to that carrying the traffic.

Suivant une comparaison abrégée des schémas d'aération usagés dans trois longs tunnels européens, on a examiné les méthodes disponibles pour aérer un tunnel véhiculaire sous-marin, long de 13 km. Il est probable que la méthode la plus propice serait une qui fournirait de l'air frais provenant d'un petit tunnel parallèle à celui où la circulation prend place.

Introduction

The Order-in-Council of Queen Victoria that joined Prince Edward Island to Canada committed the Dominion government to a "steam service for the conveyance of mails and passengers...between the Island and the mainland..." This led to several generations of ice-breaking ferries. Although service is greatly improved over that of twenty years ago, they have proven to be an expensive bottle-neck requiring periodic replacement of an increasing number of ships and of their dock facilities, and they constantly operate at a substantial loss. Successive governments have agreed that satisfactory alternatives would be acceptable under this constitutional requirement.

Alternative Connections - A railway tunnel was proposed in 1890, and tunnels have been discussed casually from time to time since then. Development of large earth-moving machines made a causeway possible. In the 1950's, investigation showed that tidal flow, harbour installations, and the fishery in the Strait would be seriously affected by a causeway. In 1968, construction began on a combination bridge-causeway-tunnel as a cure for severe traffic delays. Tenders for the beginning of the actual crossing far exceeded estimates, however, and the project was abandoned.

In the last few years the problem has been examined again. A causeway was eliminated from consideration and proposals for a "fixed link", either bridge or tunnel, were invited from organizations prepared to bid on the construction of the "link" they proposed. Several such proposals were received, in 1987. Tunnel options I and II and their variants, discussed below, are from these preliminary proposals.

The current proposals for a complete bridge largely eliminate the unpredictable effects of a causeway upon plankton, and so upon the fisheries.

A tunnel is another possible alternative. The rocks beneath the Strait are sandstones and shales, in the irregular lenticular bodies characteristic of fluvial sediments. The sandstones are water bearing. The coefficient of premeability, k, ranges from 10^{-3} to 10^{-5} cm sec^{-1} and, in preliminary design work, water flows of 18 to 24 litres sec^{-1} were estimated (Golser, 1987). The rocks are very weak (unconfined uniaxial compressive strength 100 to 250 kg cm^{-2}), so modern tunnel-boring machines should be quite practical. This weakness, however, requires that the tunnel lining be substantial and installed close behind the face as the tunnel advances. The preliminary study by Golser, in 1987, assumed cover of about 30 metres of rock between the tunnel and the sea floor; practice in similar rocks in the Sydney coal field requires 200 feet (60 metres) of cover over the workings.

Tunnel Ventilation - In a vehicular tunnel, the ventilation system must guarantee that: (1) travellers are in safe air under all traffic conditions; (2) conditions are safe for operators and maintenance staff in the tunnel; (3) in case of accident, e.g. fire, the situation can be controlled and safe conditions provided for travellers and rescue services.

The major problem is carbon monoxide. Exhaust from a modern petrol engine is about 3% CO (Innes and Tsu, 1963); diesels produce strong odours, but CO content of the exhaust is low. Consequently, the problem depends upon traffic volume, composition, and speed; upon roadway grade and upon tunnel elevation. In addition, visibility is determined by particles from diesel exhaust and from tires and, perhaps, by fog. Also, very high longitudinal air velocity may influence steering. Comfort depends upon air temperature and humidity. (Fig 1)

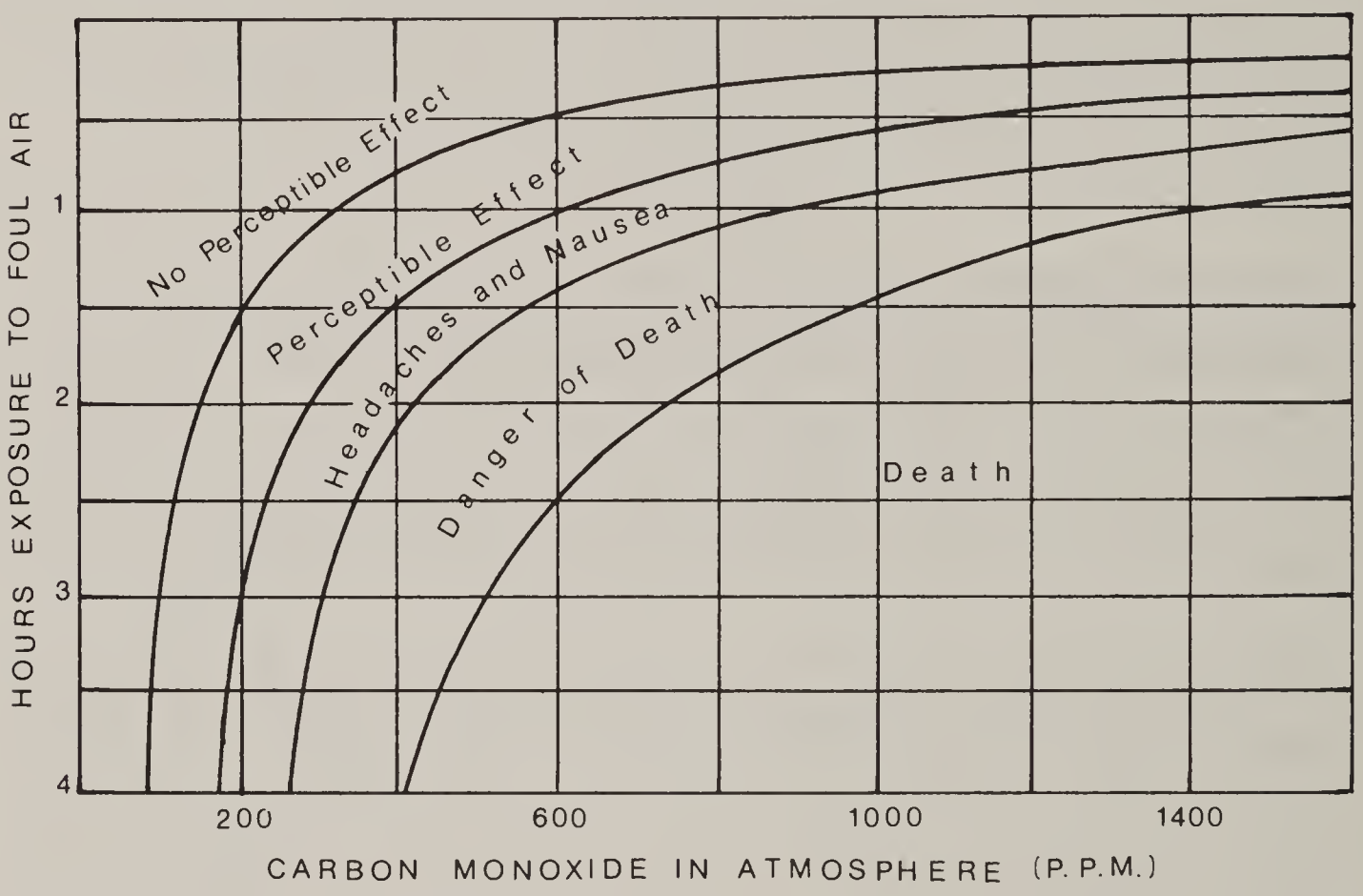


Fig 1 The effects caused by exposure to carbon monoxide.

Air Supply - Air sufficient to dilute CO to safe levels is usually sufficient to control the other factors. Common practice limits CO to $<$ 100 to 150 ppm for normal traffic, $<$ 200 to 250 ppm for congested traffic, and $<$ 50 ppm for workmen in the tunnel. Visibility is maintained by keeping particulate matter in the air to $<$ 2 to 4 mg m^{-3} for normal traffic, and $<$ 8 mg m^{-3} when congested. Longitudinal air velocity is commonly $<$ 4 m s^{-1}. (Thiéry, 1980)

In case of fire, current practice is: (1) to reduce longitudinal air movement to stop spreading of the fire and smoke; (2) to remove fumes via the exhaust airway in the roof, as immediately and completely as possible, while (3) keeping the lower part of the vehicle space free of fumes. This is done by manipulating the load on the fans. The example in Fig 2, from the Gotthard tunnel in Switzerland (Thiéry, 1980), shows the effect of stopping two sets of exhaust fans, but similar points of zero flow of fresh air result if the supply fans are suitably loaded, and the position of such points can be manipulated to some degree.

Tunnels are normally divided into ventilation zones. In the United States, the Federal Highway Administration requires that, in any zone, about 85% of total

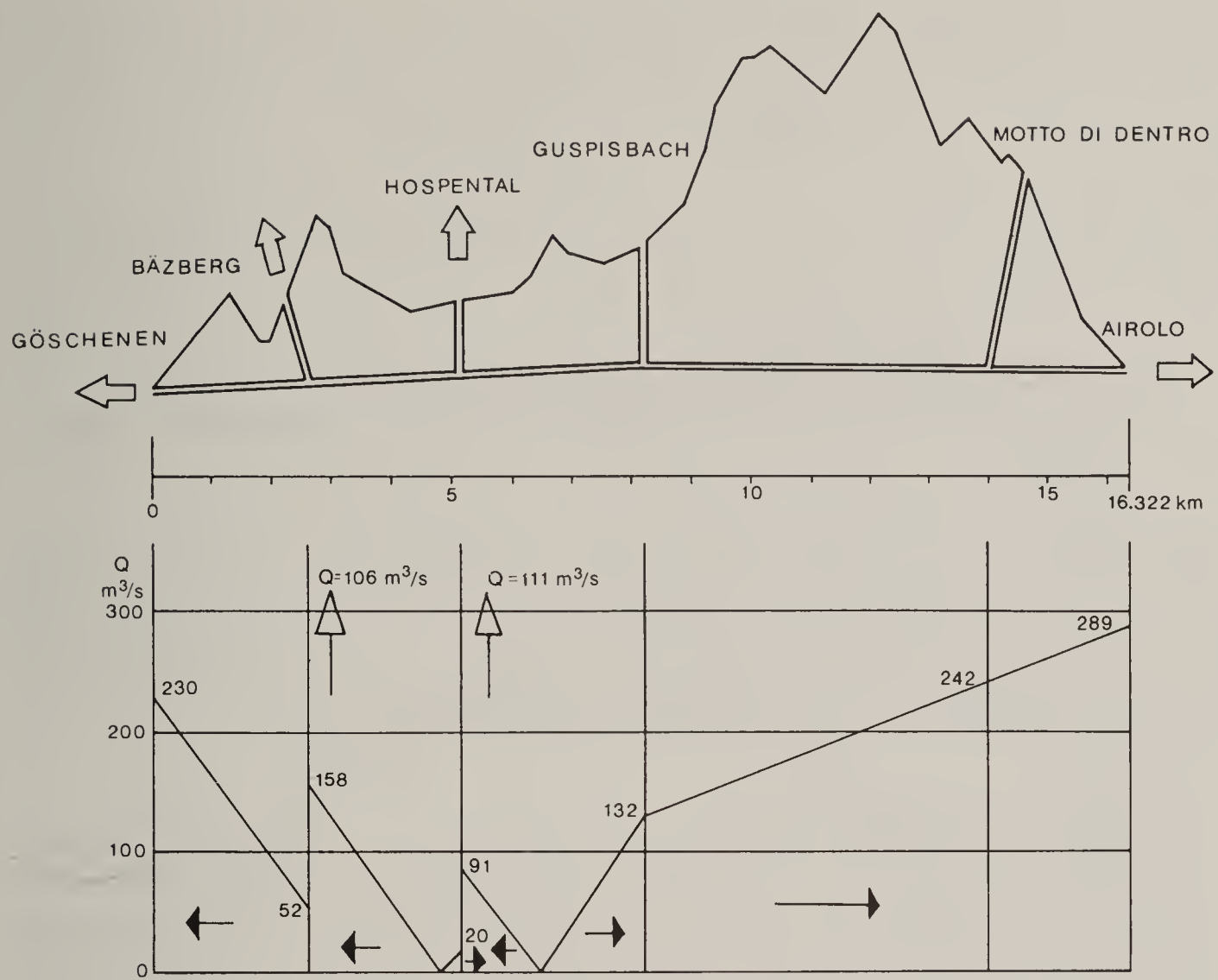


Fig 2 An example of control of air flow by manipulation of fans: the result of stopping two exhaust fans of the Gotthard tunnel. (Redrawn after Thiéry, 1980.)

capacity is maintained when one fan is out of operation (Bendelius, 1982). This can be done if four fans are employed in parallel (Fig 3).

Ventilation must be designed for extreme conditions, so size and location of airways are large factors in the design of the tunnel excavation. The pressure, P, necessary to move air in a duct of uniform cross section is

$$P = \frac{kSV^2}{A} = \frac{kSQ^2}{A^3} \text{ , because } V = \frac{Q}{A}$$

where P is in pascals, S is the internal surface of the airway in m^2, V is the air velocity in $m\ s^{-1}$, Q is the volume of air in $m^3\ s^{-1}$, A is the cross-sectional area of the airway in m^2, and k is a factor dependant upon the roughness of the airway surface, about 0.003 for smooth concrete. For a given Q, an airway of large area reduces the pressure and

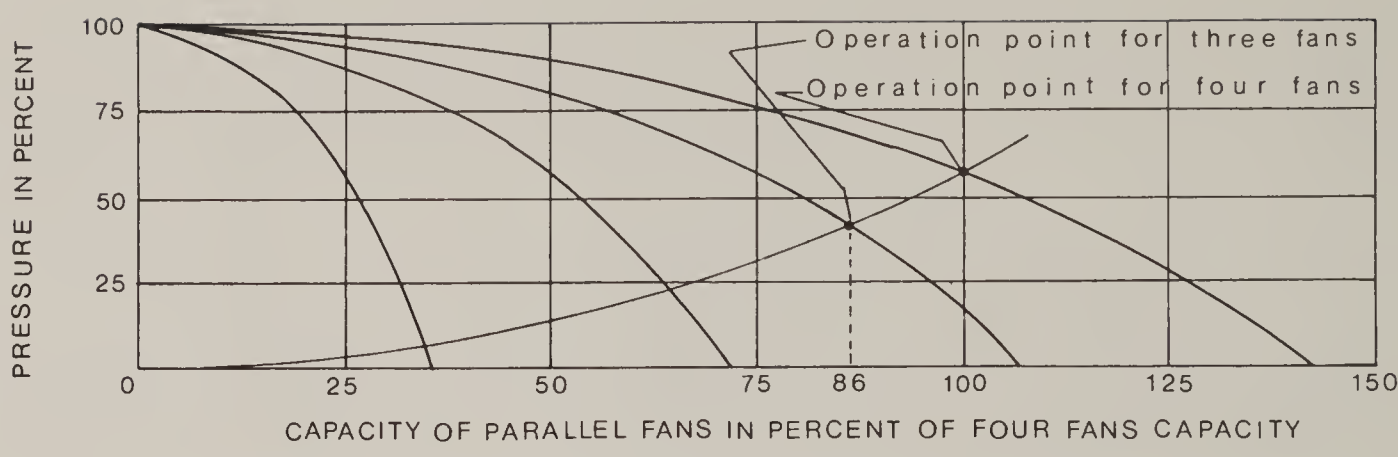


Fig 3 Operation of four fans in parallel in a ventilation zone. (After Bendelius, 1982.)

power requirements but, in a circular tunnel for example, lining costs increase as the radius, and excavation increases as the square of the radius. Therefore, although large airways produce savings in capital and operating costs for fans, the savings must be balanced against lining and excavation costs. In addition, the tunnel size, shape, and design of lining depend upon the stresses in the rocks. The inter-relations are not simple, but generally the minimum cross-sectional area is chosen.

Ventilation in Recent Long Tunnels

Arlberg, Austria (Lasser and Feizlmayr, 1978; Fig 4). At the bottom of each ventilating shaft, two fans drive fresh air about halfway to the next shaft, and two others extract foul air from the same intervals. Intake and discharge are through the same shafts, which are divided vertically. There are intake and discharge fans also at the portals. Primarily this is transverse ventilation, but some savings result from using the vehicle space as an airway, and 18% of the foul air is discharged through it. Fans are axial flow.

Gotthard, Switzerland (Diethelm et *al*, 1980; Fig 5). The mechanical arrangements follow the same system as at Arlberg, with two additional shafts. Intake and exhaust volumes are equal, i.e. fully transverse ventilation. Because of the shorter intervals between shafts, the airways in the northern part of the tunnel can be smaller than those in the southern. The fans are axial flow, and operating pressures are 1500 to 4900 pascals (Novenco Engineered Equipment). Both tunnels are divided into zones by the air shafts. In part, this provides control in emergencies but, primarily, it is to reduce the operating pressures required to move very large volumes of air. Large cross-sectional areas must be avoided because of stress from the rocks above the tunnels—up to 1000 metres of rock in both cases.

Mont Blanc, France-Italy (Ramel, 1963; Fig 6). At Mont Blanc, ventilation shafts are not possible because of permanent snowfields, glaciers and the height of the mountains above. The problem was solved by an ingenious use of supporting walls beneath the roadway to provide one exhaust channel and four inlet airways, each supplying one quarter of the north half of the tunnel. This arrangement was duplicated from the Italian portal. The scheme provides control zones, and the ingenuity lies in the increase in gallery area in each succeeding distant zone without increase in the tunnel dimensions. Half the foul air discharges through the vehicle space, with a maximum velocity of about 3.3 m s^{-1}.

All these tunnels cross mountain ranges, and differential barometric pressure between one side of the range and the other can be of concern. Longitudinal air velocity at Arlberg, for example, may be increased to 8 from the normal 4 m s^{-1}, in some circumstances.

The essential dimensions and features of the three tunnels are compared in Table I.

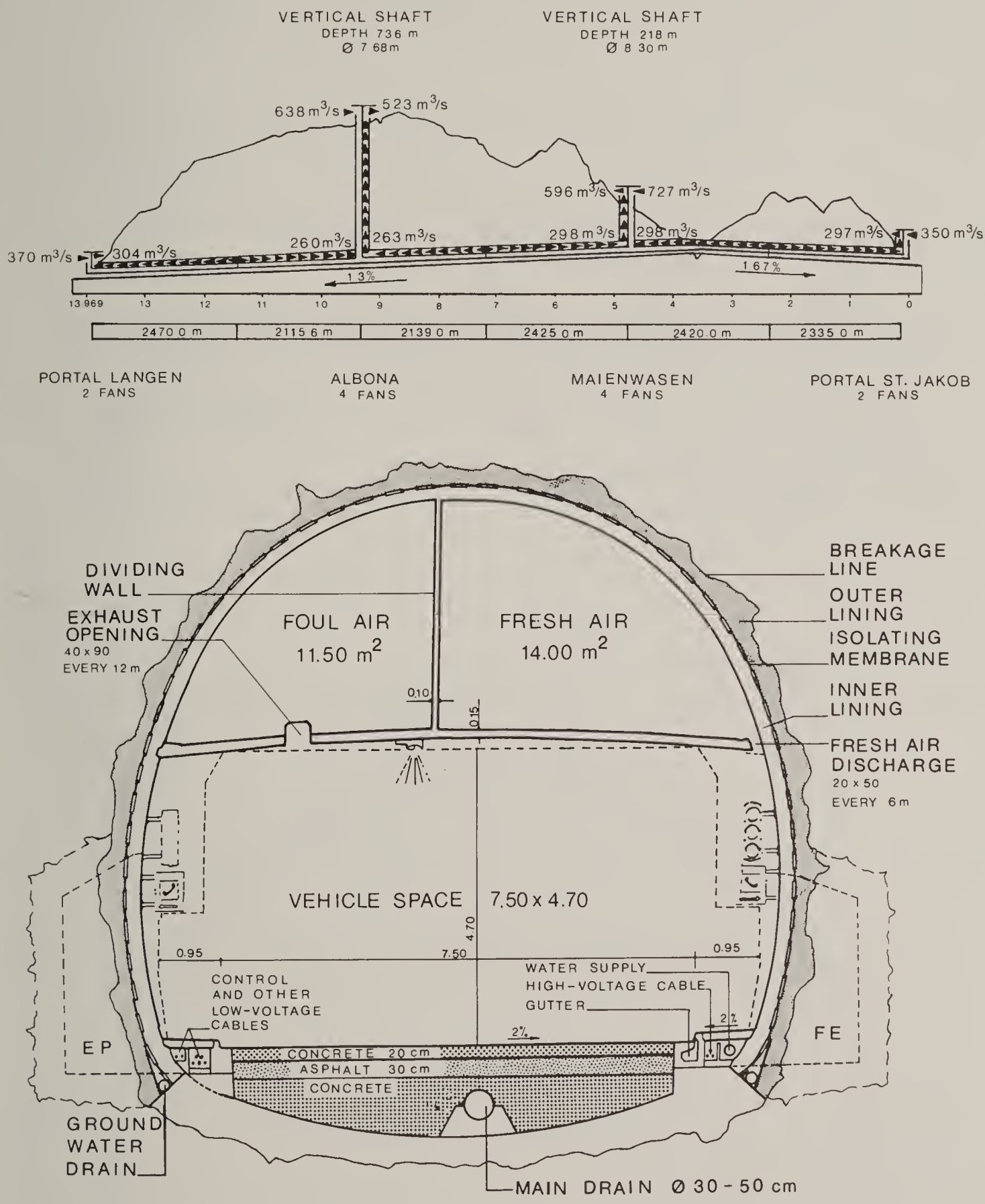
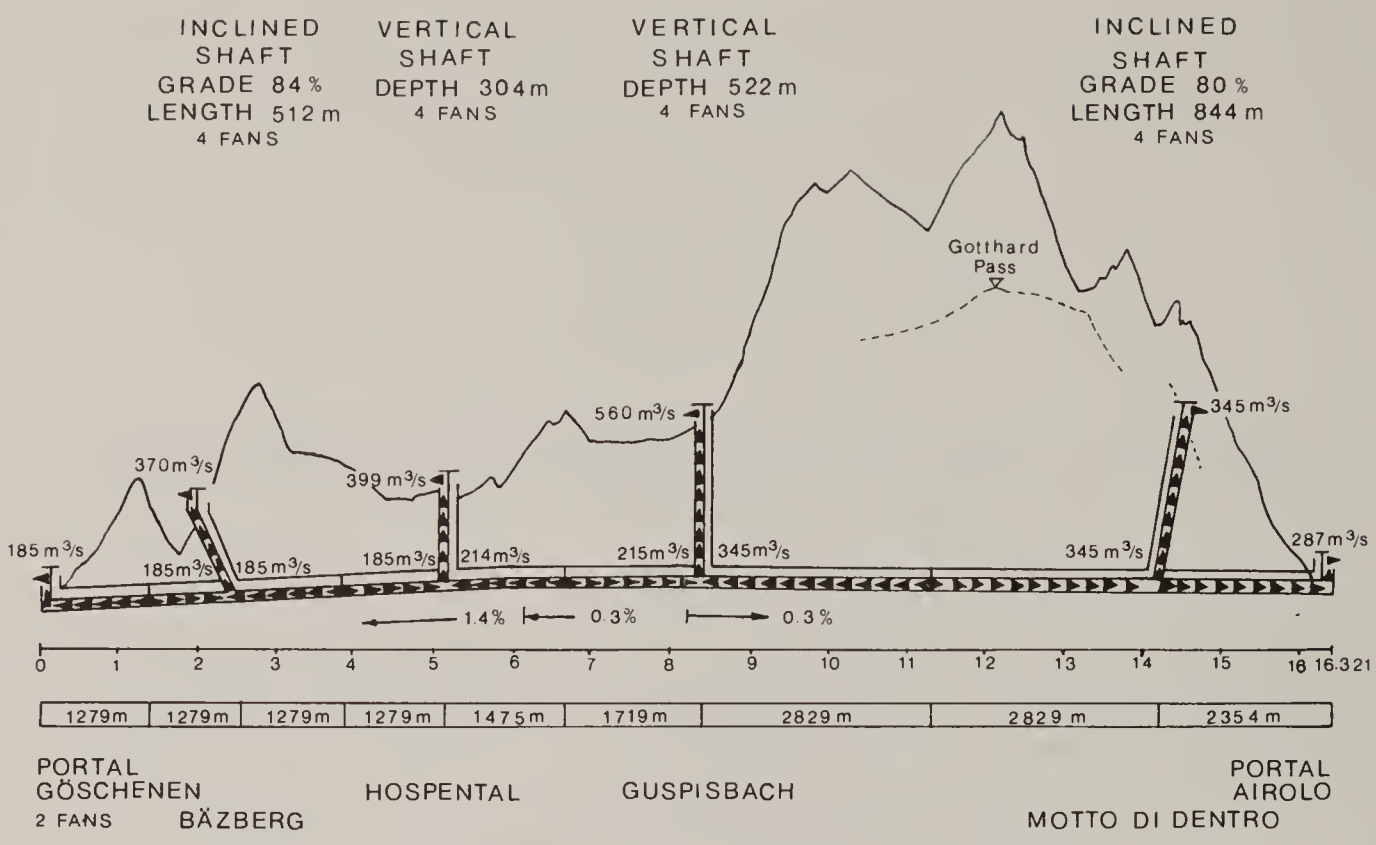


Fig 4 Ventilation scheme and typical cross-section, Arlberg tunnel, Austria. EP and FE are spaces for emergency phones and for firefighting equipment. (Redrawn after Lasser and Feizlmayr, 1988.)

Northumberland Strait

Obviously the ventilation is defined by the traffic a tunnel is required to carry. In 1927, the train ferry carried a few dozen cars each day, loaded upon railway flat cars. Probably no sensible engineer would then have predicted that, 60 years later, 145,000 vehicles would be ferried in one month. A tunnel is a very long-lasting structure,



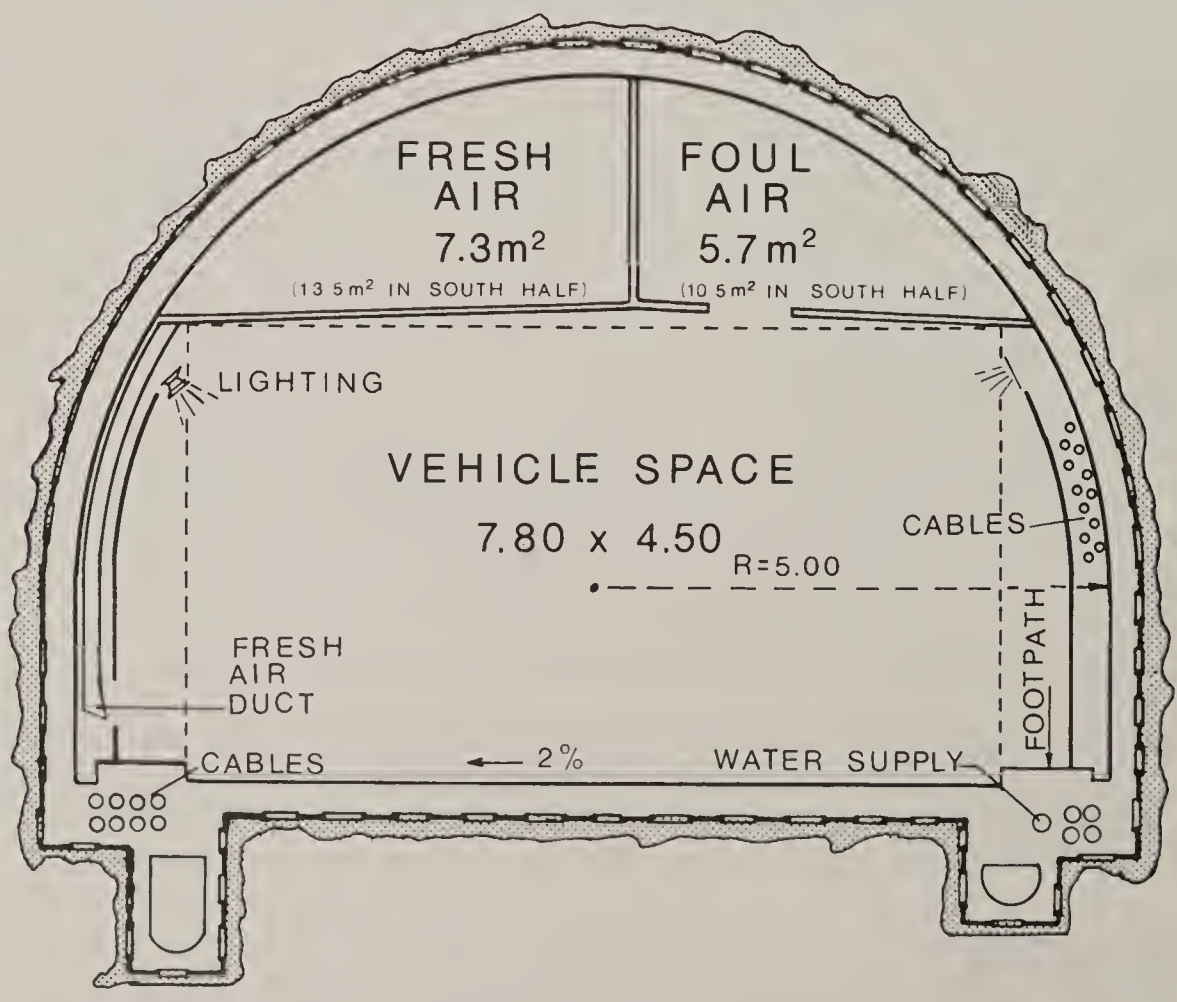
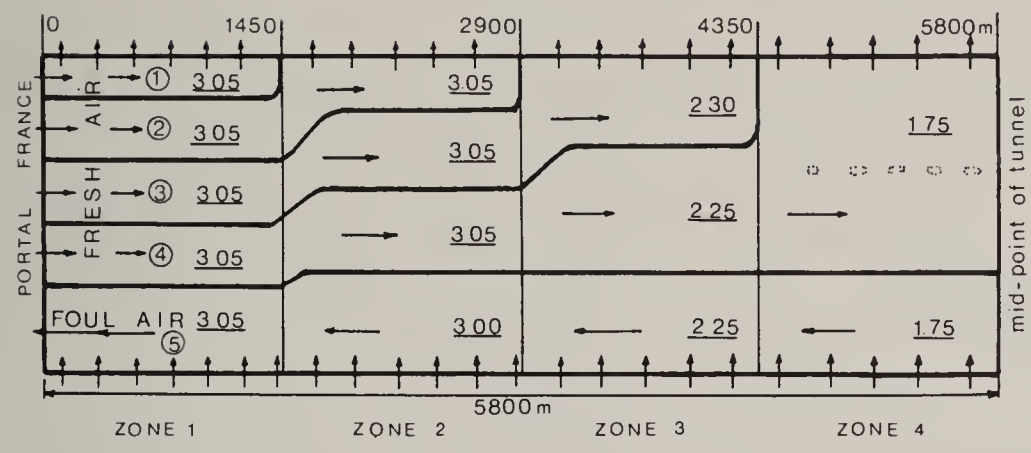


Fig 5 Ventilation scheme and typical cross-section of north part of Gotthard tunnel, Switzerland. (Redrawn after Diethelm et al., 1980.)



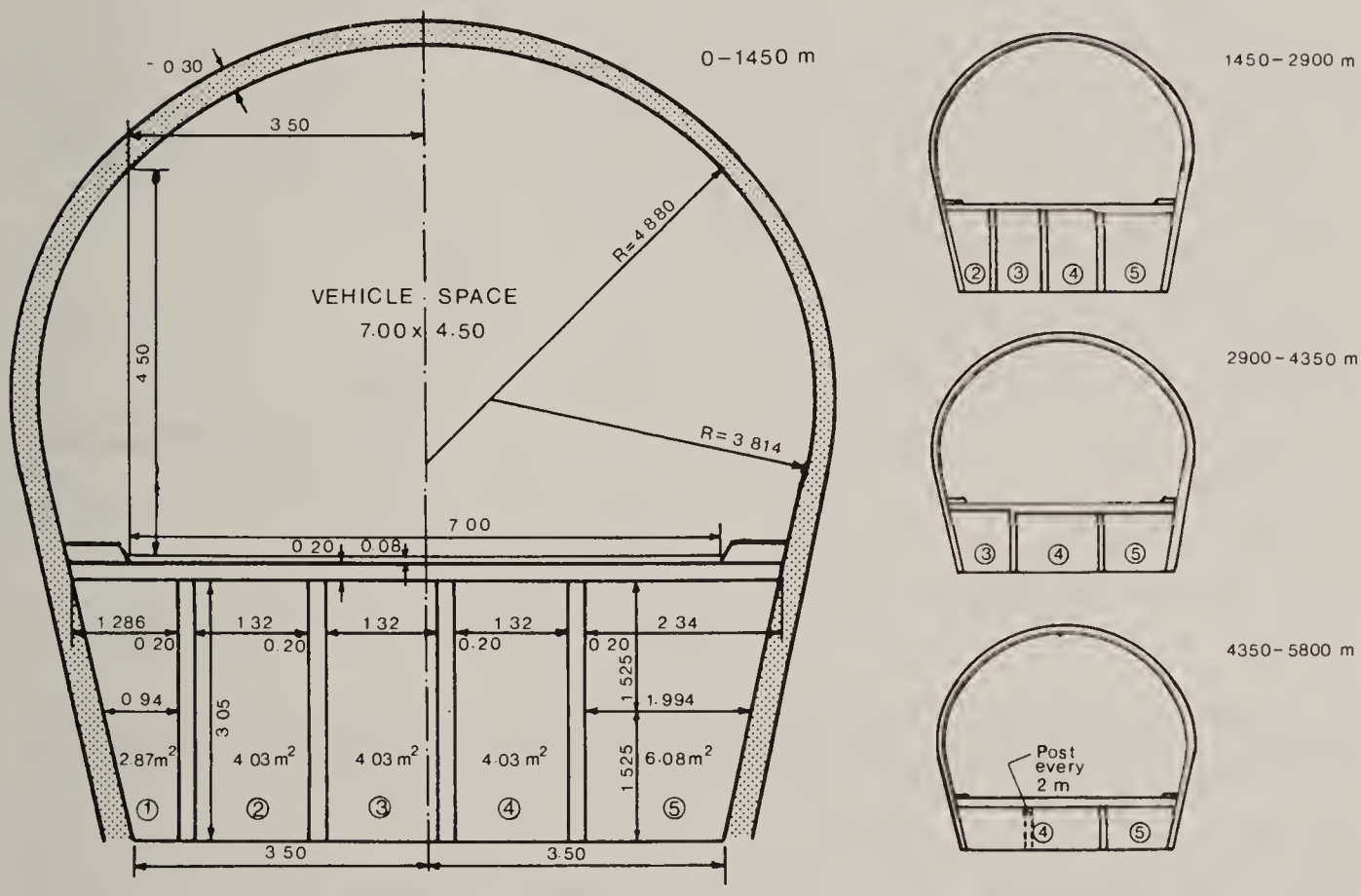


Fig 6 Ventilation scheme and typical cross-sections, north half of Mont Blanc tunnel, France-Italy. Underlined figures show height of the galleries. (Compiled from drawings supplied by Ramel.)

which cannot readily be modified while in service. Bearing in mind our imaginary engineer of 1927, and that a tunnel would probably be carrying traffic in the year 2100, if not 2200, it would be sensible to design the opening for the maximum traffic of which the roadway is capable, unless this makes the excavation and lining costs obviously ridiculous. Initial ventilation costs in "over-sized" airways would then be low, and fans could be changed later, as traffic required, without changing the tunnel itself.

Traffic Load - In the first three days of August, 1987, 26,600 vehicles were ferried across the Strait (*The Journal* newspaper, Summerside, quoting CN figures). This averages about 500 per hour for a 17-hour day.

The capacity of a 12-foot (3.66 m) traffic lane in a tunnel is approximately 2000 cars (no trucks) per hour (Bendelius, 1982). Reduction of lane width to 3.25 m reduces the capacity to 85%, and reducing lateral clearance to 0.95 m further reduces capacity to 88 percent of that figure, i.e. to about 75 percent or 1500 cars per hour. Because of trucks in the traffic stream, grades have an important effect.

Table I Comparison of Ventilation Data for Three Long European Tunnels

	Mont Blanc	Gotthard	Arlberg
Length, m	11,600	16,322	13,969
Elevation, m	~ 1200	1075-1150	850-1284
Capacity, car/hr.	450	1800	1800
heavy vehicles, %	?	10	11
Max. concentration, CO, %			
Normal	100	100	
Peak traffic		150	
Stationary traffic	200	230	
Max. grade and distance, km		1.4%-6.0	1.67%-4.0
Auxiliary shafts	0	4	2
diameter, m	-	5.60-6.65	7.68-8.30
Longest single ventilated section, m	1450	2829	2470
Area of air ducts, m^2			
Inlet	2.9 & 4.0 (min.)	7.3 & 13.5	14.0
Exhaust	6.0 (min.)	5.7 & 10.5	11.5
Longit. airflow in vehicle space, m/s	3.3	-	4 - 8
Number of fans	16	18	12
Type	centrif.	axial	axial
Operating pressure, mm H_2O	241-672	150-490	?
Total load, kW	6000	22,500	~ 11,200
Normal peak load	?	15,500	?
Total air supply, m^3/s	600	2,800	2,090
$m^3/s/km$	51.7	145	150
$m^3/s/km/veh/hr$	0.115	0.080	0.083

The Arlberg and Gotthard tunnels are designed for 1800 vehicles per hour. For comparison, the maximum traffic handled on the MacDonald Bridge, in Halifax, is 1000 cars per hour. It would be easy to jump to the conclusion that 1800 per hour is the appropriate maximum design load for Northumberland Strait, and provide the appropriate airways. A sense of proportion is needed, however: one must remember that, within a 600 km radius of the north end of the Gotthard is industrial Europe and a population of 152 million; there are another 57 million at its south end, and only a dozen major roads across the Alps.

The feasibility study released early in 1988, and prepared by Geoconsult, of Salzburg, Austria, mentions a maximum hourly traffic of 1975 vehicles and an annual average daily traffic of 9000. The recommended volume of ventilating air, however, is 80 $m^3s^{-1}km^{-1}$. For 1000 vehicles per hour this would be exactly the 0.08 $m^3s^{-1}km^{-1}$ $veh^{-1}h^{-1}$ of the Arlberg and Gotthard tunnels (Table I), and other figures in the report are consistent. Although this figure is repeated many times in other reports, it would appear that the 1975 is probably a typographical error and that the traffic intended is about 1000 per hour—double the present maximum across the Strait. Because the Gotthard ventilation apparently has proven inadequate, one might reasonably increase the air supply to 0.10 $m^3s^{-1}km^{-1}veh^{-1}h^{-1}$, and use 100 $m^3 s^{-1}km^{-1}$ and 1000 veh h^{-1} as a reasonable figure for Northumberland Strait. Certainly this is sufficient to point out the problems and the options available.

Possible Ventilation Methods

For a number of reasons it is desirable to ventilate a submarine tunnel from the land, by means of ventilation shafts near the shore at either end. The situation at Northumberland Strait is shown in Fig 7. For the location considered in the feasibility

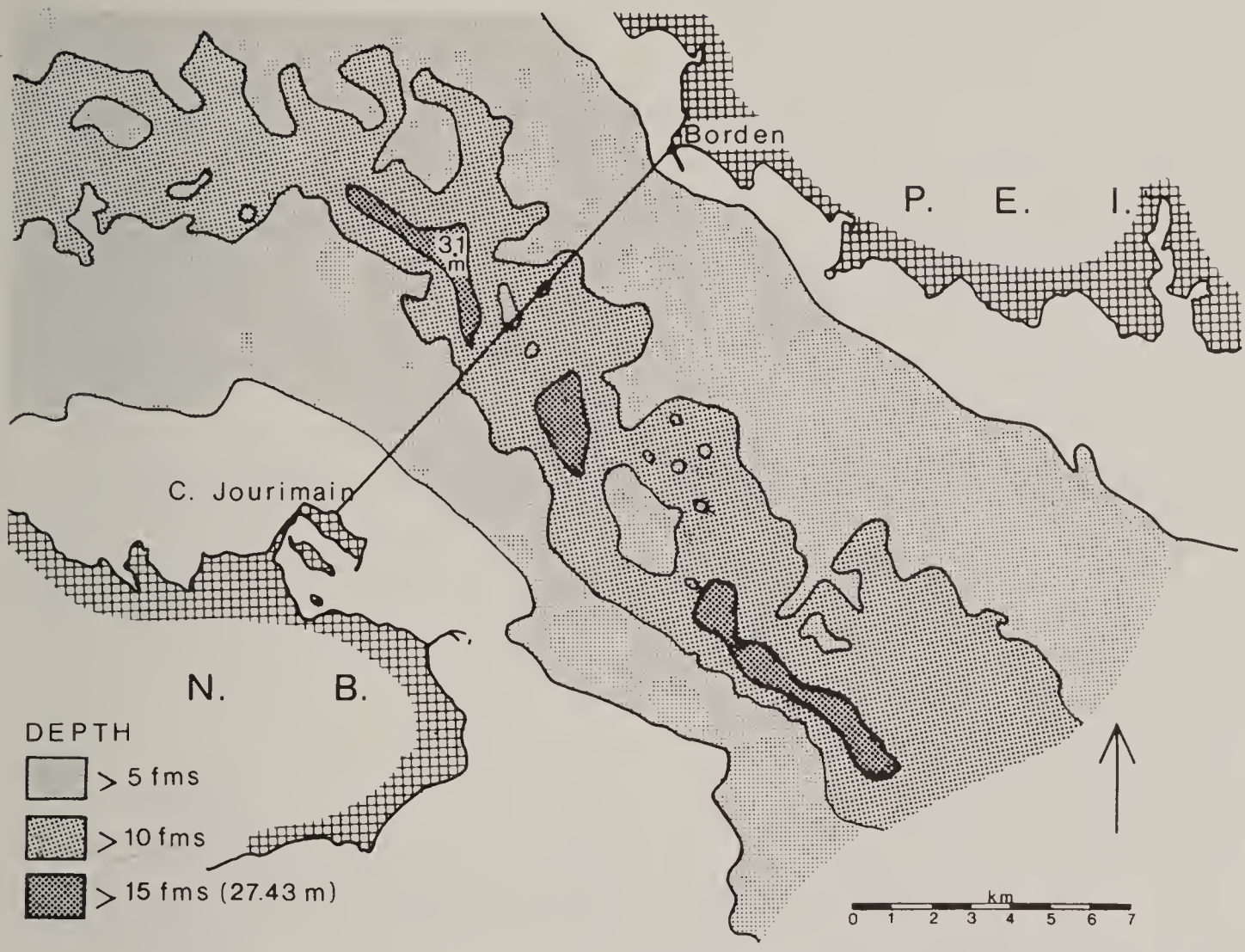


Fig 7 Water depths in Northumberland Strait, and line of proposed crossing. (Redrawn from hydrographic charts.)

study, the water gap is 13 km. At Borden, that study indicated an approach ramp 1320 m long, on 3% grade. This implies that the tunnel would cross the shore line at elevation -30 m.

The feasibility study considered a number of options. In all cases one can, for simplicity, consider that each shaft supplies air to its half of the submarine portion of the tunnel. The ramp portion, from shaft to portal at either end, is a separate and minor problem.

Option I (Golser, 1987; (Fig 8). Both intake and exhaust airways are above the vehicle space and have equal cross-sectional areas of about 18 m^2. The perimeter of each is about 17.1 m. Fresh air is discharged at regular intervals to the bottom of the vehicle space, and the foul air is extracted at intervals into the exhaust airway. If the 525 $m^3 s^{-1}$ of air required for one half (6.5 km) of the tunnel enters the fresh air channel then, after a distance of, say, 300 m an amount (300 x 525)/6500, or about 24.2 $m^3 s^{-1}$ will have been discharged into the roadway space, and about 500 $m^3 s^{-1}$ will continue into the next section of the airway, and so on. The cross-sectional area of the airway is constant, so the velocity of the air in it will decrease regularly along its length. Because the pressure required to move the air varies as V^2, however, the required pressure drops exponentially. These relations are shown in Fig 9. The total pressure required is 5200 pascals, or 520 mm, water gauge. The same situation obtains for the exhaust airway, except that the pressure is negative. The theoretical power requirement is 18,000 kW, at 60% efficiency. For this option the tunnel cross section is about 154 m^2, so the total excavation, shore to shore, is 13,000 x 154, or 2 x 10^6 m^3.

Option Ia. This differs from the above only in having the fresh air supply beneath the roadway. For the fresh air gallery the conditions are essentially as the same as in

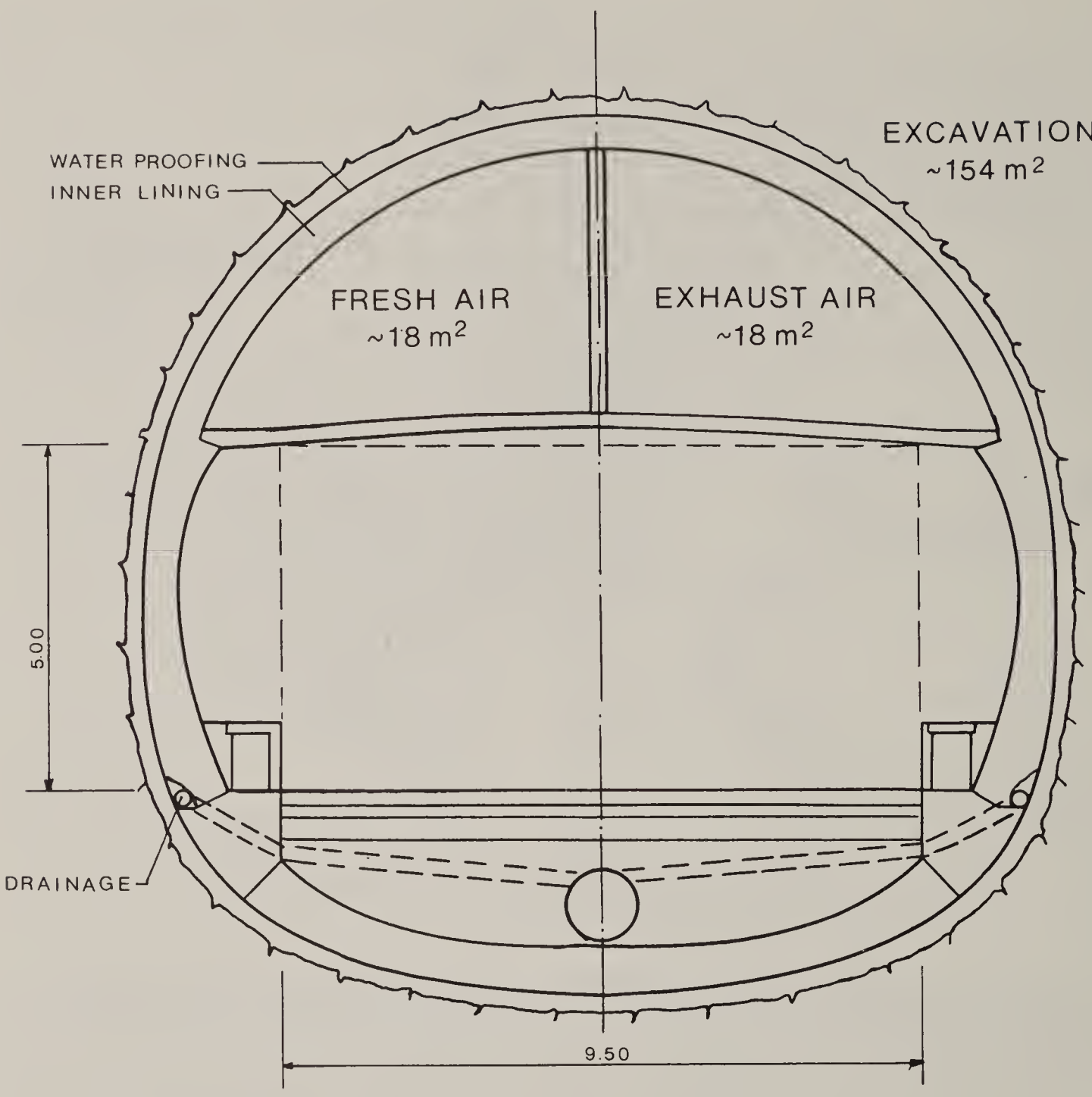


Fig 8 Typical cross-section, Option I.

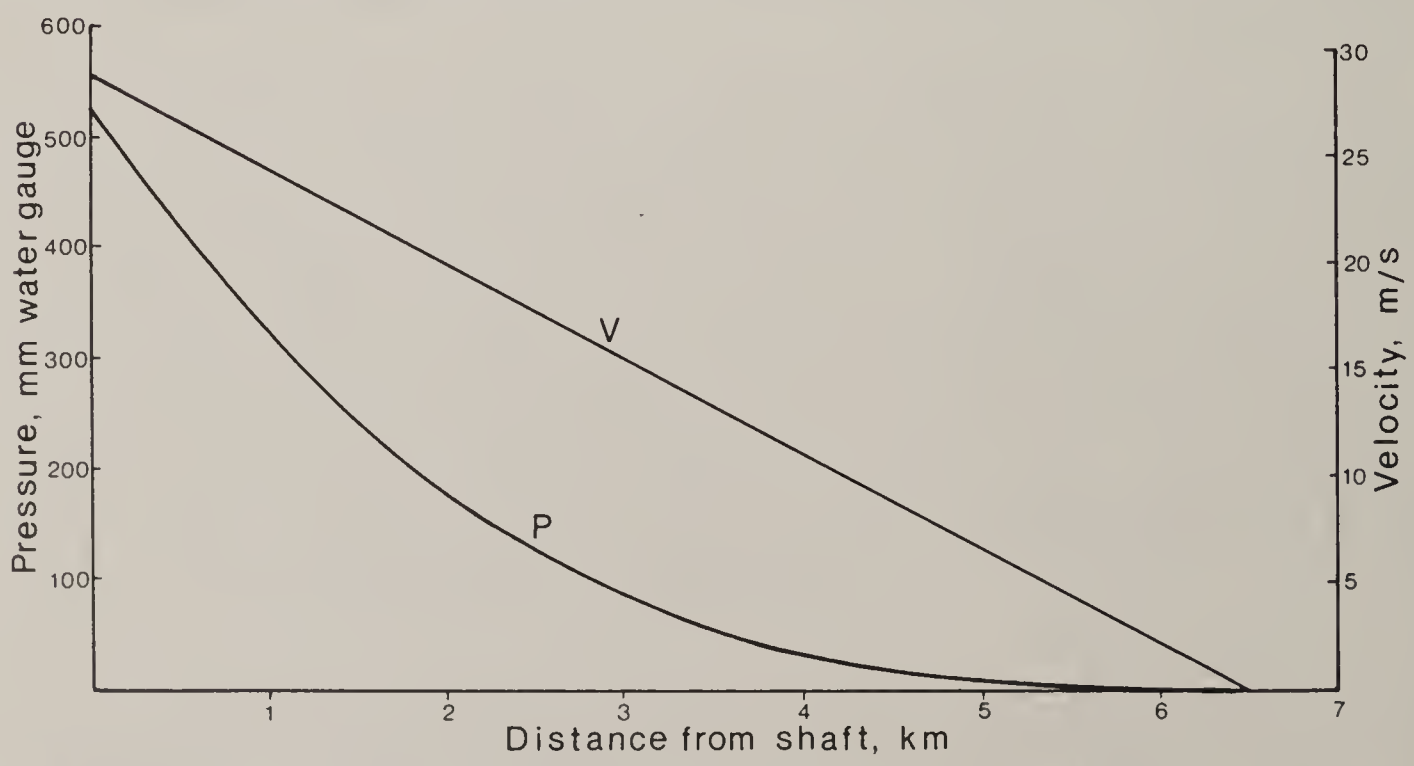


Fig 9 Pressure and velocity distribution in airways, Option I.

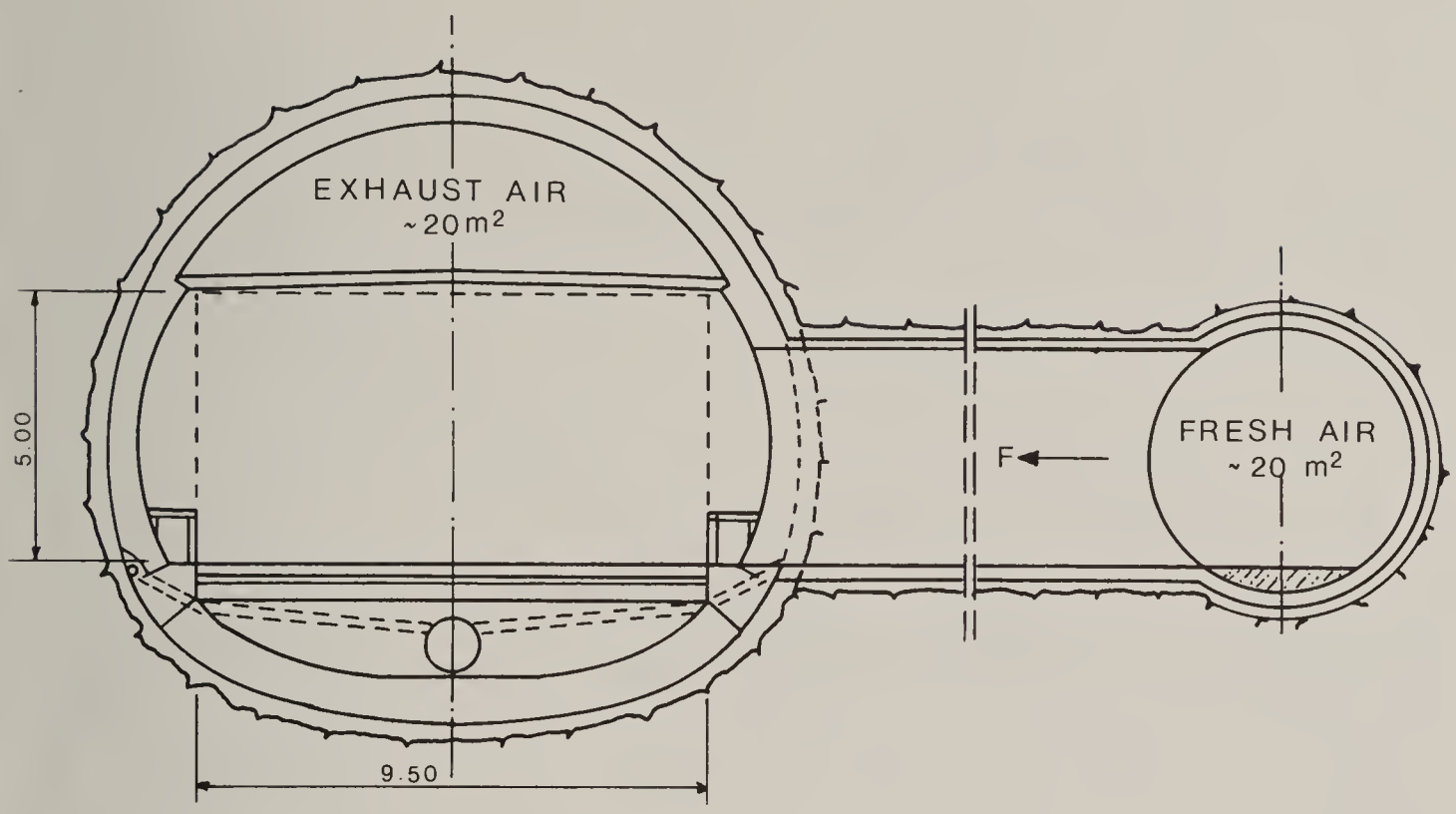
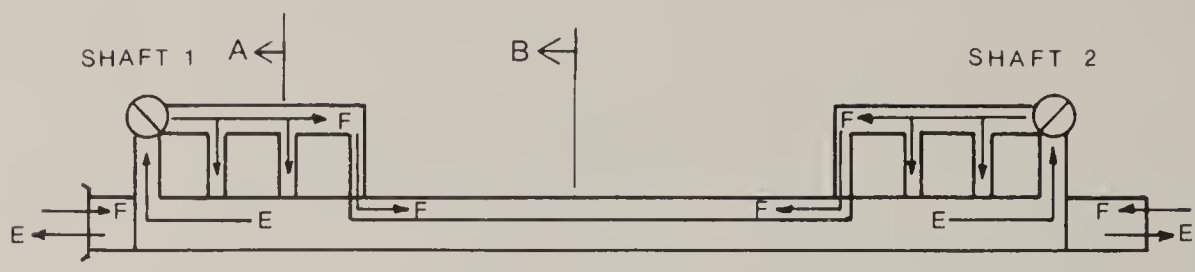


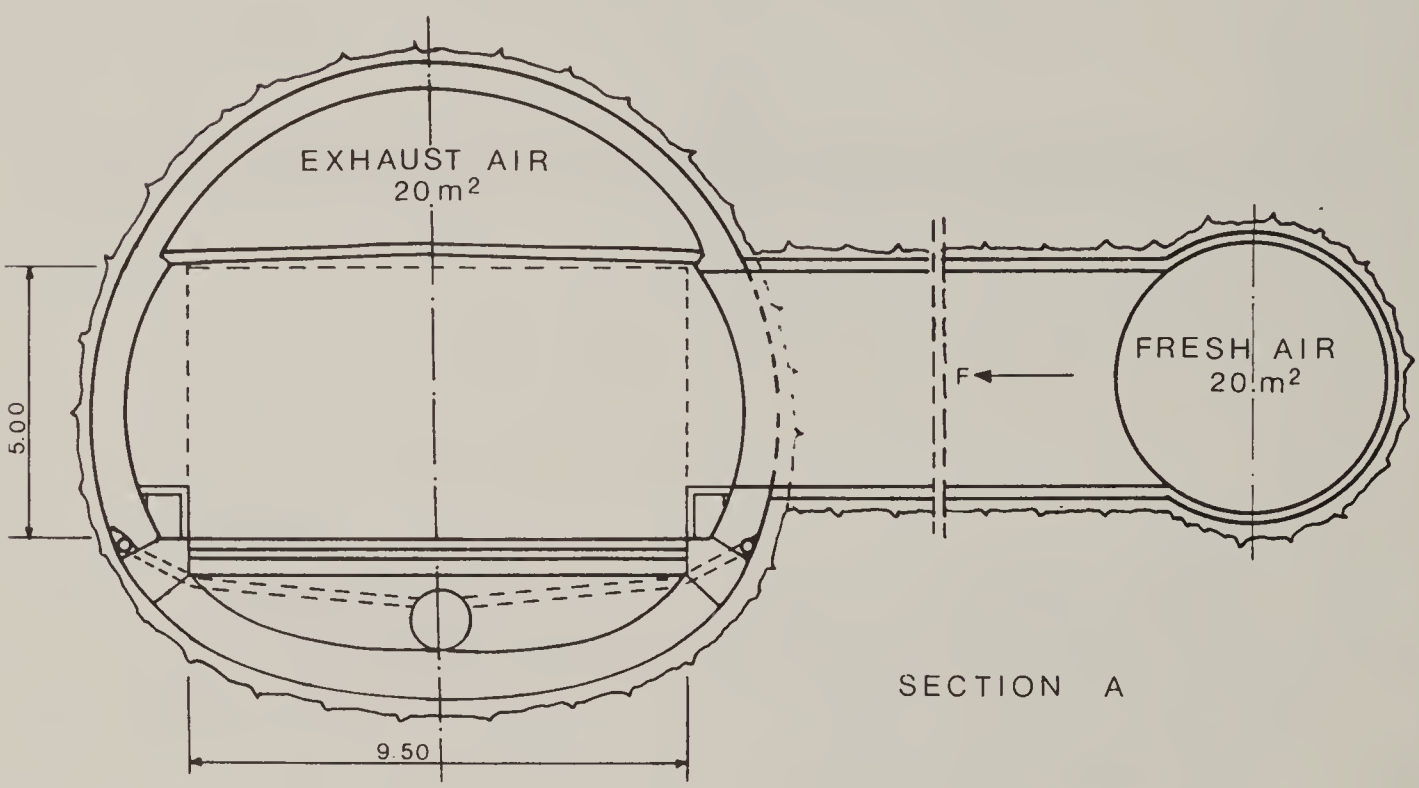
Fig 10 Ventilation scheme and typical cross-section, Option II.

Option I; the exhaust gallery, however, has a perimeter of about 21.5 m and this makes a substantial difference to the internal surface of the airway. The pressure required then becomes -620 mm, water gauge, which is a substantial change. The power requirement also increases to 20,000 kW. Because the excavated cross section is reduced to 137 m^2, the total excavation is about 1.78×10^6 m^3.

Option II (Golser, 1987; Fig 10). In this variation the fresh air is supplied from a small parallel tunnel, through connecting channels regularly spaced, and is exhausted through an airway in the roof of the main tunnel. Each airway is about 20 m^2, the perimeter of the supply tunnel is about 15.5 m, and of the exhaust airway is about 23.5 m. The pressure necessary in the supply tunnel is about 340 mm, and in the exhaust airway is about -500 mm, water gauge. The lowered exhaust pressure results from the long perimeter caused by the shape of the airway. Theoretical power requirement is about 14,400 kW at 60% efficiency. If the supply and main tunnels are on 30 m centres, as in the Gotthard, and connected by airways 4 m in diameter, at 300 m intervals, the total excavation is 13,000 (133+20)+44 (12.6 x 22), or about 2×10^6 m^3.

Option IIa (Fig 11). The *central* part of the tunnel has intake and exhaust airways above the vehicle space, as in Option I, but only 10 m^2 each. For the *end quarters* of the tunnel the whole volume above the vehicle space is used as the exhaust airway, area 20 m^2, and fresh air is supplied from a parallel ventilation tunnel, also 20 m^2 in area. This scheme avoids the problem of moving, within the main tunnel, the large volume of air that must pass through the end portions. The reduction in area of the channels beyond the end of the ventilation tunnel, however, produces an abrupt doubling of the air velocity and an attendant jump in the pressure needed to move the air. The net effect is a lowering of the exhaust pressure to -800 mm, water gauge, compared with -500 for option II, a problem for the fans. For the fresh air supply, the





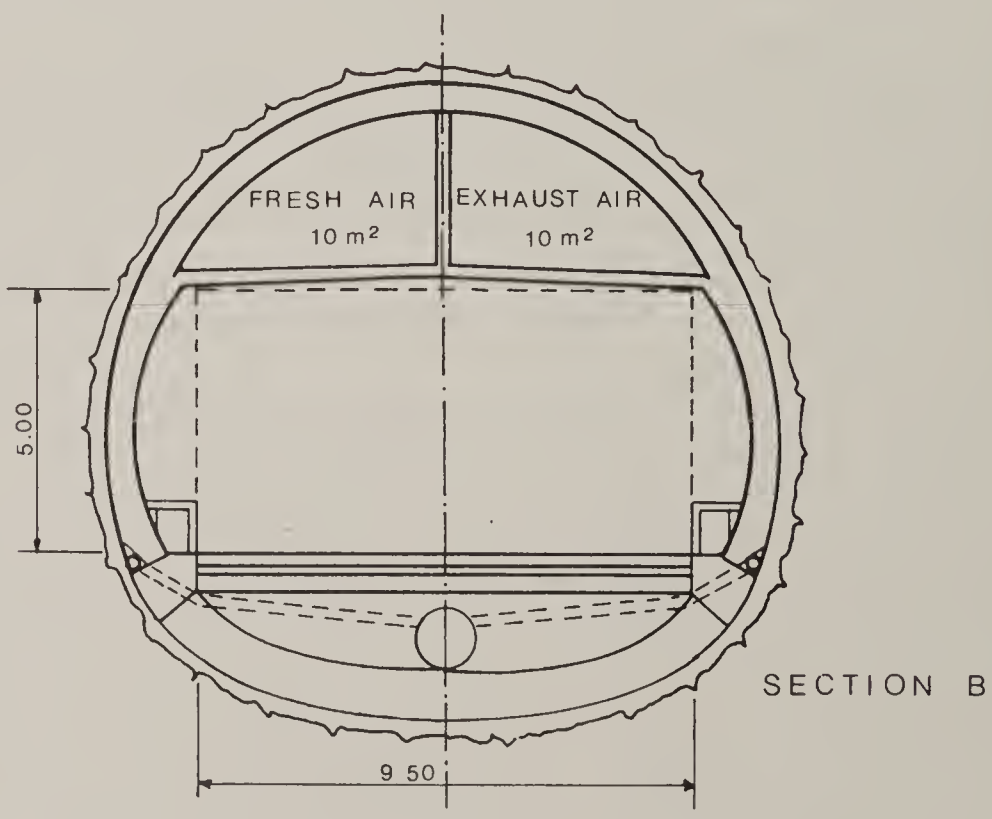


Fig 11 Ventilation scheme and typical cross-sections, Option IIa.

total pressure required is 620 mm. These features are shown in Fig 12. Theoretical power requirements are 5400 kW for the inlet and 6800 kW for the exhaust, for a total of 24,400 kW at 60% efficiency.

Option IIb (Fig 13). In this version, the air for the *central* half of the tunnel by-passes the end portion via a parallel ventilation tunnel of area 12 m². For 3250 m, then, about

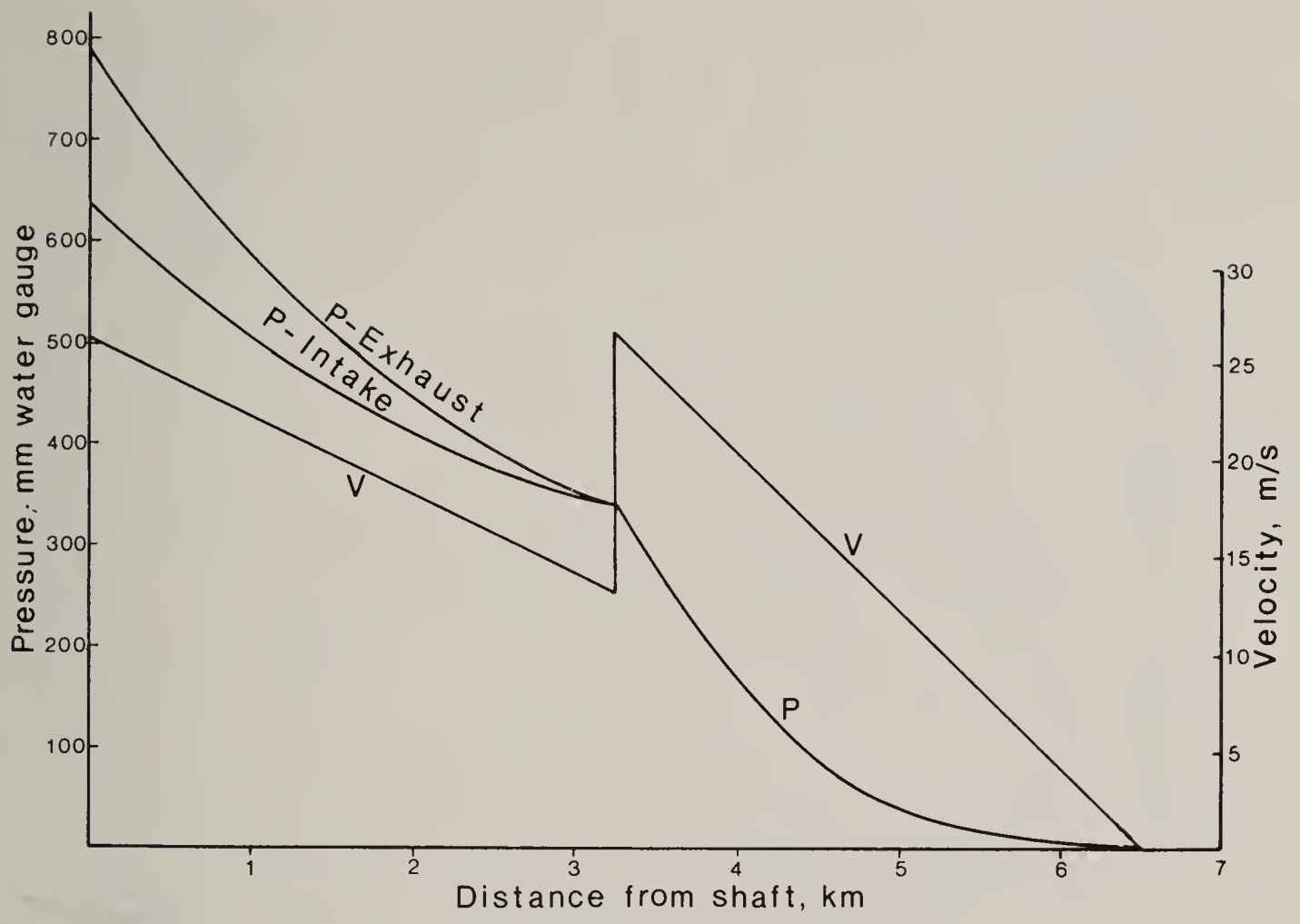


Fig 12 Pressure and velocity distribution in airways, Option IIa.

260 $m^3 s^{-1}$ moves at constant velocity in a uniform tunnel; the required pressure is 440 mm, water gauge. The air is then discharged into the vehicle space along the next 3250 m. The combined pressure requirement is 580 mm. The first 3250 m of the main tunnel is ventilated separately by a 10 m^2 duct beneath the roadway. The pressure required is 380 mm. Exhaust is by a 20 m^2 gallery in the roof, as in Option II. The pressure required is -500 mm. Theoretical power requirement is 1650 kW for the first quarter, 3000 for the 6.5 km of ventilation tunnel and 4300 for the exhaust gallery; a total of about 18,000 kW, shore to shore.

Option III (Fig 14). Although not mentioned in the recent feasibility study, it is interesting to see how the Mont Blanc system might be used under the Strait. We assume a minimum circular section to permit excavation by a tunnel boring machine. As at Mont Blanc, walls 20 cm thick support the roadway and divide the space into air galleries. Each of the four galleries supplies air to *one quarter of one-half* of the tunnel, thus generating four zones in each half of the tunnel; a fifth gallery is the exhaust airway.

As at Mont Blanc, it was found that evacuating all the foul air through the relatively small exhaust airway would cause excessively high velocities therein, and that true transverse ventilation is not practical. If one half of the foul air is discharged through the vehicle space, however, the maximum longitudinal air velocity (*ca.* 7.6 m s^{-1}) would be at the portals. This is just below the maximum 8 m s^{-1} allowed in the Arlberg tunnel but advantageously, at Borden, effects of barometric pressure are negligible.

It was found also that within a circular tunnel and a maximum fan pressure of 430 mm, as at Mont Blanc, the submarine part of the tunnel would require at least one "booster" fan in every gallery, each with the attendant special fan room. The problem, as is implied in Option IIa, above, arises from the large volume of air that must pass through the galleries in zone 1, the zone nearest the entrance.

By slashing out the bottom of the tunnel to a depth of 3.8 m below the roadway surface in the first two zones, however, and accepting the costs of fan pressures up to

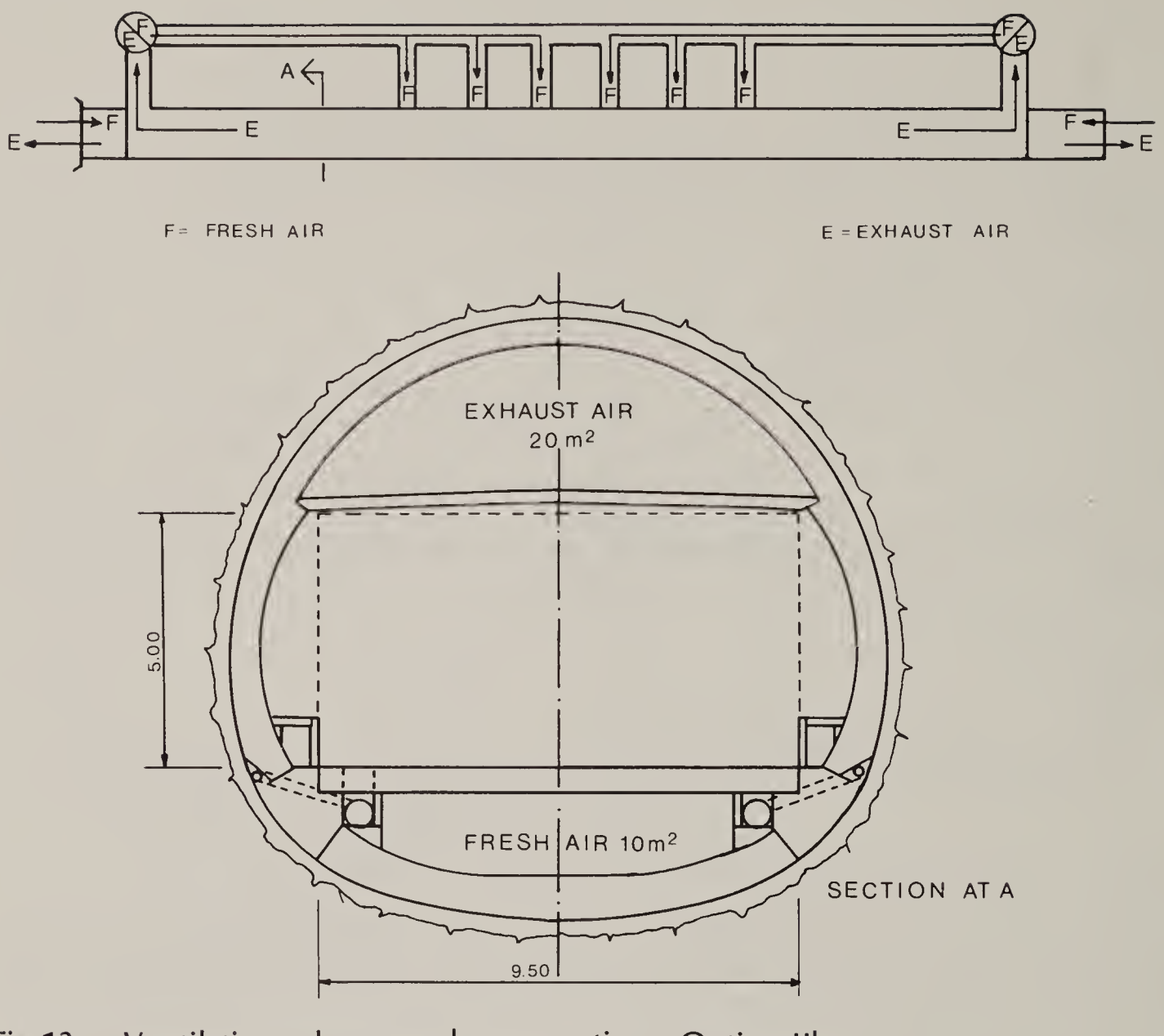


Fig 13 Ventilation scheme and cross-sections, Option IIb.

560 mm, the booster fans can be eliminated in the supply galleries. The area of individual galleries, of course, differs from zone to zone. The single exhaust gallery requires a pressure of -830 mm. The pressure and power requirements are shown in Table II. Air velocity is very high at the beginning of Gallery 1. Theoretical power requirement is 16,800 kW at 60% efficiency. Excavation amounts to about 1.5×10^6 m³.

Option IV. The system of the Arlberg and Gotthard tunnels can be applied in Northumberland Strait, if ventilating shafts, protected by artificial islands, are acceptable in the Strait. A possible scheme is illustrated in Fig 15. The minimum circular tunnel is assumed, and 45 cm allowed for the thickness of the roadway. The inlet airway beneath the road would be about 15 m² and the exhaust airway above would be about 19 m². Because of the short length and corresponding low air volume in each section, the pressure and power requirements are markedly lower than for the other options considered.

Discussion

Except for the estimates of total excavation and total power, the data shown in Table II apply to one *half* (6,500 m) of the *submarine* portion of a Northumberland Strait tunnel. The five variants of Options I and II have the dimensions used in the feasibility study by Prof. Dr. J. Golser of Geoconsult, consulting engineers, of Salzburg, Austria, and air volume of 80 $m^3 s^{-1} km^{-1}$ as in that study. Options III and IV assume 100 $m^3 s^{-1} km^{-1}$. The pressure and power requirements were calculated by a

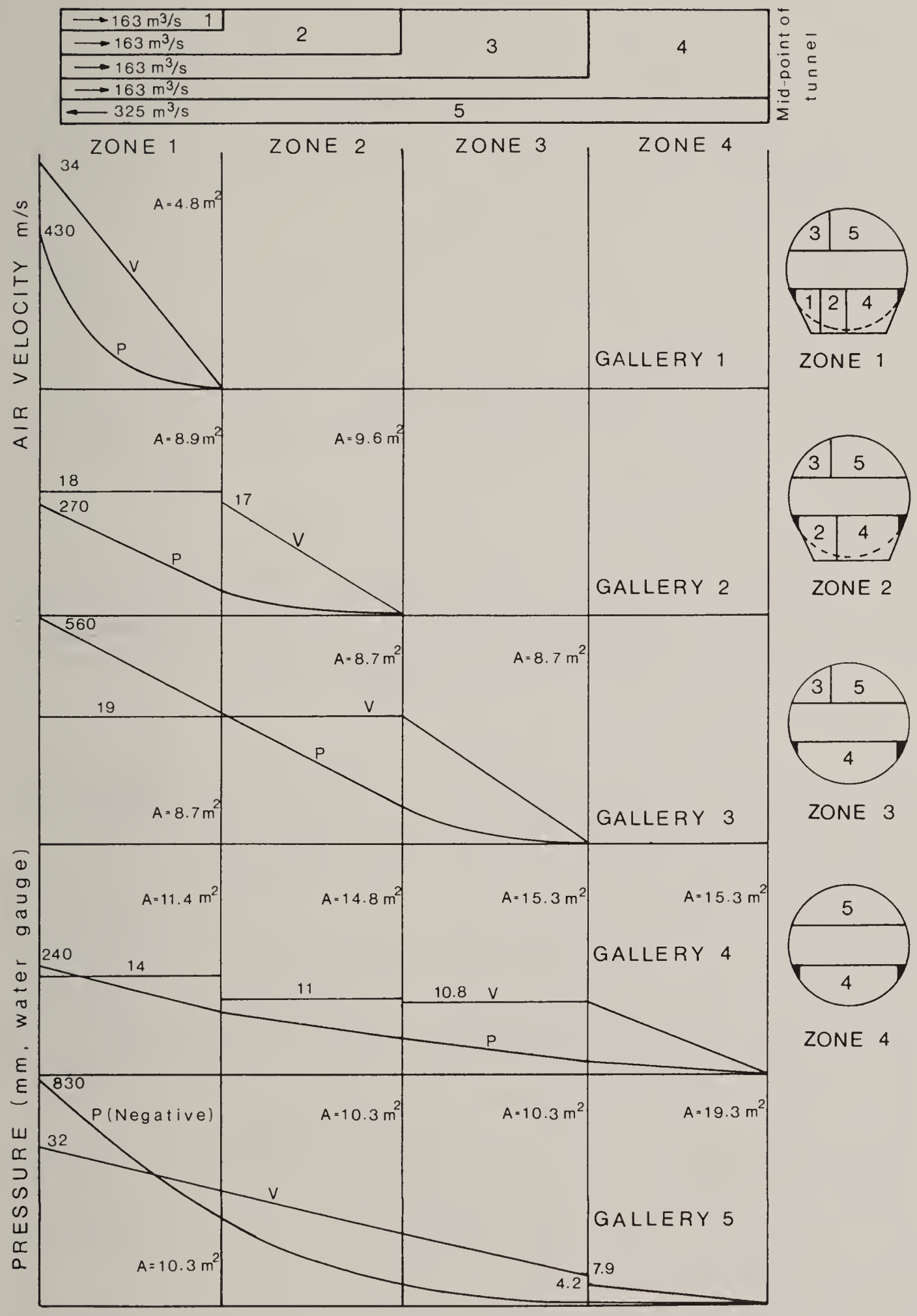


Fig 14 The Mont Blanc ventilation scheme applied to a Northumberland Strait tunnel. The invert of the tunnel is slashed to increase the cross-sectional area of the galleries in zones 1 and 2.

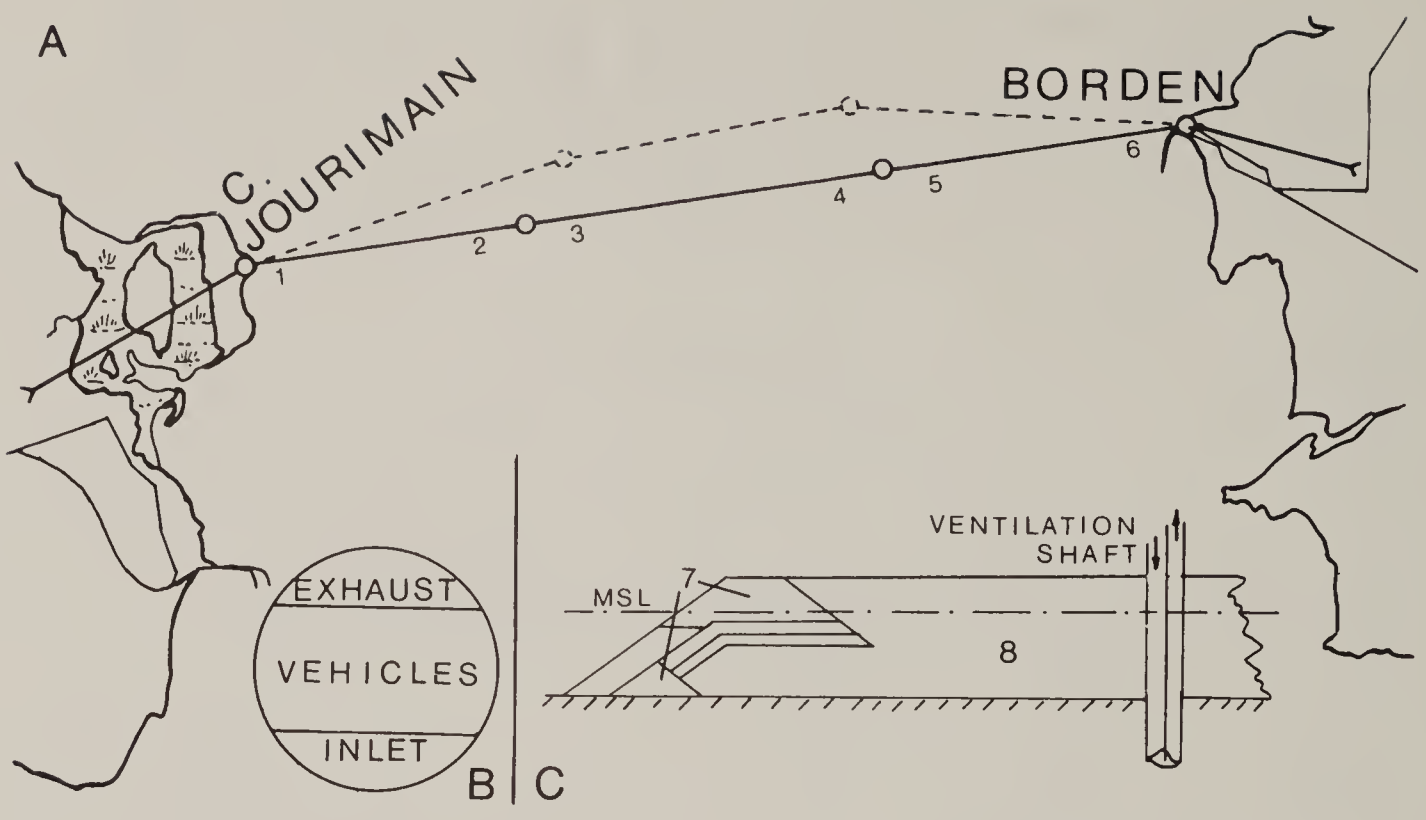


Fig 15 Option IV. A: Possible position of ventilation shafts in the Strait. 1-6, ventilation zones in the tunnel. The dashed line is an alternative position which would put the shafts through exposed rock bottom. B: Diagram of tunnel cross-section. C: Cross-section of protective island. 7, armour stone, 5 kg to 7 tonnes; 8, rock spalls and consolidated sand.

mechanical integration, using the above basic formulae and considering the airway in 100- or 300-metre increments. If the results are valid as a first approximation, they show a substantial range in the pressure and power requirements.

The pressure is probably the most critical factor. Axial flow fans can move enormous volumes of air, at pressures usually less than 50 mm, water gauge. As pressure increases the construction tolerances become important, and costs increase accordingly, but even 20 years ago axial flow fans capable of 500 mm pressure were in colliery service in France. The Danish firm that supplied the fans for the Gotthard tunnel informs me that they have supplied fans with capacities over 400 $m^3 s^{-1}$ at pressures greater than 800 mm, or even much more, and that some of them have now been in service for more than ten years (Novenco, 1988). Axial flow fans with variable pitch rotors are desirable because of ease of adjustment and the possibility of reversal of the air flow.

By placing fans in series, either immediately adjacent to one another or at intervals in the airway, the total pressure capability is increased two, three, or more times. It must be remembered, however, that a series fan placed well along the tunnel must have a special fan room, with hoisting equipment, and that replacement of major parts may be very difficult.

The airways must also respond to the air pressures and velocities. Losses due to friction-induced turbulence must be kept to a minimum, of course. In addition, in Option I for example, the maximum pressure difference between the airways is 2 x 520 mm, which translates to 1040 kg m^{-2} on the surface of the dividing wall. This is several times the force normally allowed as wind load in building design. Obviously separation of the airways requires more than hanging a curtain between them! A similar situation occurs in Option IIa, where the pressure difference between the exhaust airway and the vehicle space is 800 mm, or 800 kg m^{-2}.

Table II Data for a Northumberland Strait Tunnel under Various Ventilation Options

Option	I*	Iα*	II*	IIa*		IIb*		III+					IV+		
INLET AIRWAYS				1st	Main	1st	Vent.								
Gallery or Zone No.				Quarter	Tunnel	Quarter	Tunnel	1	2	3	4	5	1+2	3+4	5+6
Length, m.	6500	6500	6500	3250	3250	3250	6500	1625	3250	4875	6500		1900	2500	2100
Area, m²	18	18	20	20	10	10	12	4.8	8.9	8.7	11.4		15	15	15
Perimeter, m	17.1	18.5	15.5	15.7	14	16.5	12.6	9.5	12.1	12.6	13.3		17.5	17.5	17.5
Max. Air Velocity, m/s	29	29	26	26	26	26	22	34	18	19	14		13	17	14
Max. Pressure, mm, w.g.	520	550	340	620	325	380	580	430	270	560	240		41	89	54
Theor. Power[1], 60% eff., kW	4500	4700	2900	5400		1650	3000	1150	720	1480	640		130	360	180
EXHAUST AIRWAYS															
Area, m²	18	18	20	20	10	20						10.3	19	19	19
Perimeter	17.1	21.5	23.5	23.5	14.0	23.5						13.7	26.4	26.4	26.4
Max. Air Velocity, m/s	29	29	26	26	26	26						32	10	13	11
Max. Pressure mm, w.g.	-520	-620	-500	-800	-325	-500						-830	31	66	41
Theor. Power[1], 60% eff., kW	4500	5300	4300	6800		4300						4400	96	270	140
Total Power[2], kW	18000	20000	14400	24400		18000		16800					2400		
Excavation x-section, m²	154	137	133+20	133+20	133	133	12	111					111		
Total Excavation[2], m³x10⁶	2.0	1.8	2.0	1.9		1.9		1.5					1.5[3]		

* Air supply: 80 m³/s/km.
\+ Air supply: 100 m³/s/km.
[1] For one-half of tunnel only.
[2] Shore to shore, excluding approach ramps.
[3] Excluding shafts.

In Option III, walls subdividing the tunnel invert provide support for the roadway, which reduces the stresses within it, and so its thickness and cost. In zone 4, posts are required to fill this duty.

Option II has some features not immediately apparent. If the parallel ventilation tunnel is driven first, it provides a positive check upon all rock and hydraulic conditions for the main tunnel, and may act as a drain to relieve pressure in any aquifers encountered. It can also be used to provide access, should grouting or other procedures be needed to control water or bad ground in advance of the main tunnel. Detailed knowledge of conditions should reduce the contingencies for unknowns which contractors include when bidding on a job. In the Gotthard tunnel, and presumably in this one, refuge rooms (Schutzräume) for about 60 persons are spaced at intervals of about 250 m along one side of the tunnel. If they are suitably placed, an assured air supply for these rooms can come directly from the ventilation tunnel, which is also available as an emergency tunnel.

In Option IV, the two additional ventilation shafts are not minor engineering problems, are expensive, and are probably politically impossible at present. First, although producing negligible effects upon tidal currents, and so upon the fishery, there is the question of obstructions in a navigable waterway. Second, they must be stable. Preliminary examination 30 years ago indicated that protective islands 200 m in diameter at the top would not be disturbed by the moving ice. Since then, much design experience has become available from drilling operations in the Beaufort Sea. Third, such an island, in 20 m of water, with sides sloped 1 : 1.5 covers an area of about 53,000 m^2. Its sloping sides have an area of about 26,000 m^2, for a net loss (?) of lobster habitat of about 50 per cent.

The shaft itself might well be a concrete caisson sunk through the island, and sealed into the bedrock beneath; the portion in the bedrock is a standard mining operation. The portion above the bedrock would be expensive, but the islands do provide a space for the disposal of spoil from the tunnel. Two islands would require about 2 x 10^6 m^3, of which about half would be rock spalls; the balance is armour stone, which is available at Port Hastings.

Without fairly reliable figures for excavation costs, it is not possible to infer the most suitable tunneling scheme. The low power and excavation requirements of Option IV are probably completely offset by the matter of shafts in the Strait. Option III also has relatively low power and excavation requirements, and roadway costs would be somewhat less than where the entire road is not supported from beneath. If a circular tunnel is adequate to sustain the rock and hydraulic pressures, excavation by boring is likely to be less expensive than by standard drill and blast procedures. Although excavation is at a maximum in Option II, power requirements are comparable to those of Option III and the engineering and safety advantages of the parallel ventilation tunnel may well be considered to offset the costs of excavation. It is probable then, that Option II would be the one likely to be chosen.

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References

Bendelius, A.G. 1982. *Tunnel Ventilation in* Tunnel Engineering Handbook (Eds. J.O. Bickel and T.R. Kuesel), Van Nostrand Reinhold Co., New York, pp. 483-563.

Diethelm, W., Gallati, F. and **Henke, A.,** 1980(?). Darstellung des Projektes [Gotthard]. *Schweizer Ingenieur und Architekt,* 36/80, pp. 803-822.

Golser, J. 1987. Northumberland Strait Crossing, Tunnel Feasibility Report, Preliminary Study, Geoconsult Consulting Engineers, Salzburg, Austria. (For Dept. Public Works, Canada).

Innes, W.B. and **Tsu, K.** 1963. *Automobile Exhaust Control in* Encyclopedia of Chemical Technology (ed. Kirk-Othmer), 2nd Ed., vol 2, Interscience Publishers, John Wiley and Sons, New York, p. 828.

Lasser, P. and **Feizlmayr, A.** 1978. Vorbereitung, Planung und Bauleitung für den Arlberg Strassentunnel. *Österreichische Ingenieur-Zeitschrift,* Nov. 1978, pp. 373-394.

Novenco Engineered Equipment, Naestved, Denmark. Correspondence, Feb. 1988.

Ramel, F. Ingenieur en chef des Ponts et Chaussées, 1er Arrondissement, Département du Rhone, France: Correspondence, Jan. 1963.

Thiéry, J-P. 1980(?). Le Système de gestion de la ventilation [Gotthard]. *Schweizer Ingenieur und Architekt,* 36/80, pp. 882-886.

(Received 16 December 1988).

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Obituary of C.R. Masson 1922 - 1988

During the decade 1967 - 1976 noteworthy contributions to our knowledge of silicon and sulphur chemistry, to techniques of determining the biosynthesis of natural products and to elucidation of their complex structures were made at the National Research Council's laboratory in Halifax. C.R. Masson, treasurer and long-time member of the Institute, who died on1 2 March 1988, played a major role in this extraordinary efflorescence. He was born on 8 September 1922 in Aberdeen and graduated from Aberdeen University in 1943. He pursued graduate studies with H.W. Melville, was awarded a Distiller's graduate scholarship and took up the investigation of molecular weight determinations of polymers by osmometry. This work initially involved modifications of the osmometer designed by Fuoss and Mead (1943), which notably included an effective thermostating device, the use of cellulose membranes biosynthesised by *Acetobacter xylicum* and careful attention to the manipulation of the membranes produced by this organism. The resulting instrument was used to determine the osmotic pressure developed in a number of e.g. toluene solutions of common commercially available polymers and in some cases the values of the Huggins "constant" in these systems. In the cases of samples of polyvinyl acetate in ethyl acetate it was shown that the molecular weights obtained by osmometry agreed well with those calculated from polymerisation kinetics. These results were later elaborated when the molecular weights of a series of vinyl acetate polymers of viscosities in the range 0.5-2.15 were examined by osmometry and by a light scattering method which gave values in general agreement in the range of 1-15 x 10^5. The Rayleigh scattering method also provided information on the polymer shapes and this was used to explain discrepancies in the molecular weights obtained by the two methods. Several years later Masson used this experience in studies of the viscosity and degradation kinetics of the naturally occurring sulphated polysaccharides carrageenin, work of considerable contemporary interest in the light of the role these materials play in ulcerative colitis (Marcus, *et al.* 1989).

Free radical chemistry

In 1948 Masson came to Canada to work with E.W.R. Steacie in the National Research Council's laboratory in Ottawa. He became associated with Steacie's long standing interest in the photodecomposition of simple organic compounds in the gas phase with photoexcited ($M(^3P_1)$) mercury, cadmium and zinc species. Steacie and Masson studied the photodecomposition of propane and methyl chloride and in the latter case were able to delineate the stoichiometry of the reaction and to make suggestions for the process at pressures < 100 mm i.e.

$$Hg\ (6^3P_1) + CH_3Cl = Hg\ (6^1S_0) + CH_3\cdot + Cl\cdot$$

and chart the subsequent reactions of the radicals. The discerning reader of these papers will appreciate the amount of work done to produce these results and will note the observation of the sensitivity of $Cl\cdot$ to the presence of traces of oxygen. Masson's interest in photochemical processes, initiated by lines in the mercury arc spectrum, continued when he moved into Noyes laboratory as the C. & H. Dreyfuss post-doctorate fellow at the University of Rochester, New York. There he studied the photolysis of heptan-3-one in the gas phase and confirmed earlier observations by Bamford and Norrish (1935) that the process $(C_3H_7)_2CO \rightarrow C_2H_4 + CH_3COC_3H_7$ occurs. He also demonstrated for the first time that the radical reaction: $(C_3H_7)_2CO = 2C_3H_7\cdot + CO$ is important and he found evidence for 7 other products, mostly from further reactions of $C_3H_7\cdot$ many of which he identified. This elucidation of the photochemis-

try of simple aliphatic ketones is a considerable tribute to Masson's chemical insight and experimental skill. He continued these studies after he moved to the National Research Council's laboratory in Halifax reporting on measurements of the quantum yield of CO on photolysis of di-isopropyl ketone and on further investigation of the rate of association of n-propyl radicals.

By 1965 experimental evidence was accumulating (Richardson, 1962) that confirmed the suggestions of Flood and Forland (1947) and Richardson (1955) that silicate melts were mixtures of anionic species $Si_nO_{3n+1}^{-(2n+2)}$. Armed with this information and the theoretical work of Toop and Samis (1962), Masson proposed, on general thermodynamic grounds, a relationship for the distribution of species in silicate melts that depended on the postulated equilibrium constant of the reactions:

$$M_2SiO_4 + M_{n+1}Si_nO_{3n+1} = M_{n+2}Si_{n+1}O_{3n+4} + MO$$

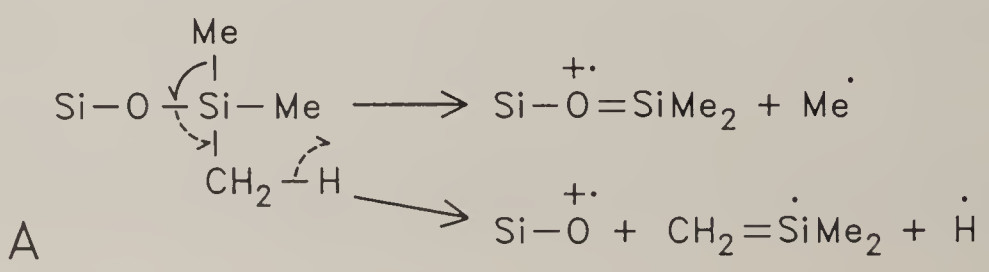
which was therefore only dependent on the nature of the cationic species M. Using published data Masson showed that the calculated relationships fitted the experimental results for binary systems e.g. $CaO\text{-}SiO_2$, $CoO\text{-}SiO_2$, $NiO\text{-}SiO_2$ etc., well. It followed that many other thermodynamic properties of these and related systems could be predicted, including average molecular weights. Most of the assumptions in the development; some plausible, others, e.g. the absence of intramolecular reactions, less so, were similar to those used by Flory (1941) in his development of an expression to describe the distribution of oligomers derived from monomers of the type AXB_2 where A and B are different functional groups. The two procedures led to conclusions that differed in detail — conclusions of doubtful validity because of the simplifying assumptions made in both cases. The proposals of Masson and his colleagues led to some controversy, but much more importantly, initiated new experimental work on the chemistry of silicates and ultimately greater understanding of the properties of metallurgical slags.

Chemistry of silicates

Lentz (1964) had demonstrated that the rection,

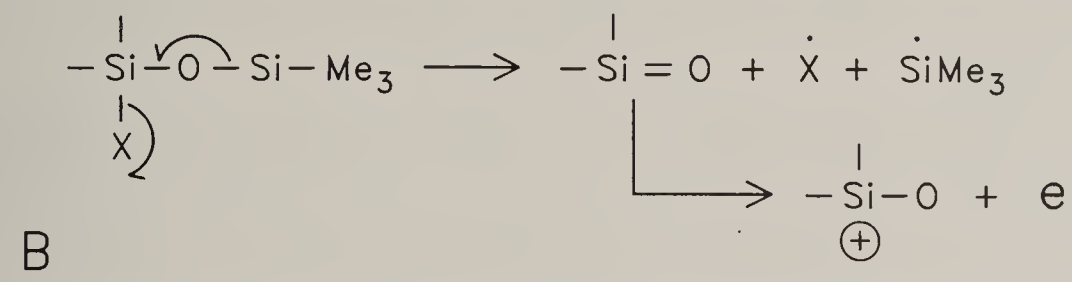
$$M_{n+1}Si_nO_{3n+1} + (2n+2)\ Me_3SiCl = Si_nO_{3n+1}\ (SiMe_3)_{2n+2} + (n+1)MCl_2$$

proceeded in aqueous solution, in heterogeneous systems and that the products were volatile. Masson undertook a detailed investigation of this reaction, work typical of the elegant experimental techniques that characterized all he did. Thus hemimorphite ($Zn_4(OH)_2Si_2O_2{\cdot}H_2O$) under the conditions used by Lentz gave the trimethylsilyl derivatives of SiO_4^{-4}, $Si_2O_7^{-6}$, $Si_3O_{10}^{-8}$ and $Si_4O_{12}^{-8}$ in the proportions 29:27:15:29 and Masson demonstrated that the time and temperature of the reaction and the proportion of hydrochloric acid in the reaction mixture initiated reactions in the silicate before trimethylsilylation. Based on these observations a reaction protocol was developed that produced partially silylated entities as intermediates, the reaction being completed in the presence of an Amberlyst ion exchange resin. This resulted in hemimorphite being converted into its hexatrimethylsilyl derivative with less than 4% trimethylsilylated byproducts. Structural information about the trimethylsilylated silicates was obtained by mass spectroscopy. The trimethylsilyl ethers were collected as they emerged from the chromatography column in an ingenious trap, in capillaries that could be directly inserted into the electron impact source of the mass spectrometer. It was shown that the ions reactions:



A

were of high probability; the ions of highest molecular weight undergoing at least the first of these fragmentations. Studies with fluorosilicates also showed that the process:



B

where X = F or $OSiMe_3$, for example occurred with high probability (There are a number of errors in J. Chem. Soc. (Dalton Trans.) 1979, 457; e.g. Figs 4 & 5 are transposed). This work provided information on the purity of the samples under study, their molecular weights and the presence or absence of fluorinated species. This mass spectroscopy greatly extended the use of the technique in inorganic chemistry, though mass spectrometer sources used for such purposes had a short life.

The analogous reaction: $M_{n+1}Si_nO_{3n+1} + (2n+2)Me_3GeCl$ was also studied and its products, the derivatives $Si_nO_{3n+1}(GeMe_3)_{2n+2}$ characterized in the same manner. However, the yields obtained were not so good e.g. $SiO_4(GeMe_3)_4$ was obtained in 80% yield from calcium orthosilicate, and further exploitation of this interesting variant was not pursued.

The technique of trimethylsilylation was exploited in several directions. In mineralology notable contributions to our knowledge of the structures of olivine, forsterite, tephroite, fayalite, andradite, laumontite and dioptase were made. Spectacularly, it was shown that olivine was present in lunar samples collected (lunar latitudes and longitudes) at 1°N 25°E (Apollo 11); 0°S 22°W (Apollo 12) and at 5° 30′S 17° 30′W (Apollo 14), one of the first demonstrations of the extraterrestrial applicability of the law of constant proportions. The technique was also used in some interesting aspects of soil mineralology, e.g. an analysis of imogolite, an aluminium silicate with an unusual tubular structure. This work showed that useful information could be obtained on intractable non-crystalline solids and semi-solids, e.g. cement paste, and was therefore ideally suited for investigations of the compositions of metallurgical slags and glasses.

Metallurgy

In 1954, insufficient research and development work was being done in Canada on the chemistry of metallurgical processes despite the economic importance of the industry. In response to recommendations by a National Research Council Associate Committee and by the chairman of the British Iron and Steel Research Association, a small group of physical chemists was created to work in this field and Masson was asked to lead the group. For the purpose of this review of Masson's work, it is convenient to divide the metallurgical studies that ensued into two parts; that dealing with gas-liquid metal interactions and that dealing with the thermodynamics of metallurgical slags. Of course both lines of work were closely intertwined, especially in the experimental techniques that Masson and his collaborators devised and developed to study processes occurring in the temperature range 1000-2000°C.

Studies of gas-metal systems

Rates of reactions are of great monetary importance in the commercial operation of metallurgical processes, and in steelmaking the most important rate is that of decarburization of iron-carbon melts. In his early metallurgical work Masson studied this rate, as well as the rate of desulphurization of such liquid alloys, and showed that they were controlled by diffusion of reactants or products in the gas phase. A novel

electrochemical cell was used to follow the kinetics of the dissolution of oxygen into liquid silver, where the rate determining step was shown to be diffusion in the liquid metal. Such kinetic research was taken up by a colleague and continued for many years as an important part of the work of Masson's group.

In his systematic study of gas-metal interactions, Masson first examined the thermodynamic activity of the metal oxide component of $MO\text{-}SiO_2$ melts. One technique for measuring such activities required determining the small (0.001-0.02%) amounts of oxygen that would dissolve in a metal phase in equilibrium with the slag. These techniques involved considerable experimental difficulties and were subject to errors, and Masson carried out a great deal of work to improve the existing methods e.g. vacuum fusion (Sloman, 1959) and isotope dilution (Kirshenbaum and Grosse, 1952) were carefully compared. Analogous investigations into the determination of oxygen in titanium at 1850°C by isotope dilution were reported.

The thermodynamic reference for the activity of FeO was the solubility of oxygen in iron when the latter was in equilibrium with pure FeO. The latter was difficult to obtain — crucibles tend to dissolve in FeO at 1600°C! Masson developed a levitation method which got rid of the necessity to use a crucible, and then pushed the reference solubility up to the hitherto unapproachable temperature of 1960°C.

Constitution of Metallurgical Slags

In the 1950's questions were being directed towards the constitution of metallurgical slags in order to rationalize their behaviour as e.g. desulphurizing and dephosphorizing agents towards liquid iron alloys. What was a molten calcium silicate slag like at the molecular level — what were the bonds, what were the aggregations? Masson began to answer some of these questions by applying physical chemical techniques at high temperatures. For example he used the method of depression of freezing point, with molten alkali and alkaline earth fluorides as solvents, to measure the molecular weights of silicate slags. The experiments were difficult, and required ingenious solutions. Masson prepared the purest calcium fluoride then known by the bold expedient of bubbling anhydrous hydrogen fluoride through molten calcium fluoride at 1300°C, the equipment being made of graphite in the hot zone and teflon in the cold.

Another high temperature adaptation of a standard physical chemical method was the use of EMF cells. In the late 1950's Masson used magnesium oxide as an oxygen electrode to measure the oxygen potential of open-hearth slags. This was pioneering work which anticipated by several years the German development of doped zirconium oxide as a thermodynamically acceptable oxygen electrode material. For this work Masson developed an ingenious double-compartment concentration cell slip cast and sintered out of magnesia. This marked the beginning of a line of research in ceramics at the Atlantic Research Laboratory.

While polymer-ionic theory of slag constitution was being developed, Masson and his collaborators carried out a wide range of experiments to determine the polymerization constants for various systems. These methods depended on finding the thermodynamic activity of the metal oxide component of silicate and silico-phosphate melts. In some experiments slag and metal phases were equilibrated, with the oxygen content of the metal giving a measure of the activity of metal oxide. These experiments were carried out in specially selected and fabricated ceramic crucibles, as well as by levitation.

In other types of experiment an EMF cell was used to measure activities. Both oxygen (doped zirconia) and fluoride (CaF_2) electrolytes were utilized. A great deal of work was done on the lead systems $PbO/SiO_2/P_2O_5$ and $PbO/PbF_2/SiO_2$, partly stimulated by the lead-silver development in New Brunswick. This work included activity measurements of plumbous oxide in $PbO/SiO_2/P_2O_5$ solution with up to 12.5

mole % P_2O_5 in the temperature range 1050-1350°C. It was shown that substitution of silica by phosphorus pentoxide reduces the activity of plumbous oxide and leads to increasing the negative partial heats of mixing of the oxide. The solubility and transport of oxygen through and from these melts and evidence that the reaction:

$$2Pb^{+2} + O_2 = 2Pb^{+4} + 2O^{-2}$$

occurred in these slags were also studied in detail. Finally this work was extended to the system $PbO/PbF_2/SiO_2$ and details of the equilibria between the various silicate and fluorosilicate species were delineated to the point that the value of 0.4 ± 0.025) of the equilibrium constant for the reaction series:

$$F^- + Si_nO_{3n+1-m}\ F_m^{-(2n+2-m)} = Si_nO_{cn-m}\ F_{m+1}^{-(2n+1-m)} + O^{-2}$$

was estimated for basic melts. These studies utilized electrochemical methods where the crucible slip-cast from calcium fluoride was used as the solid electrolyte (F^- being solely responsible for conduction) between the $PbO/PbF_2/SiO_2$ and PbO/PbF_2 half cells. In many ways this work paves the way for the preparation of pure compounds of the type $Si_nO_{3n-m}F_{m+1}X_{2n+1-m}$ i.e. a completely novel series of inorganic compounds.

The foregoing sketch of 40 years of scientific effort by Charles Masson does scant justice to his achievement. Belatedly this was recognized by the Alcan award of the Canadian Institute of Mining and Metallurgy and by his election to the Royal Society of Canada. Browsing through his list of publications one is aware of their international flavour. Some of this work was done in laboratories outside the National Research Council and conversely many post-doctorate students of Masson can now be found doing productive work in the United States, Europe and the Far East. Those of us who were his colleagues over a more extended period are aware of our loss and we extend our sympathies to his wife and family.

References

Bamford, C.H. and **Norrish, R.G.W.** 1935. Primary photochemical reactions. Part VII. Photochemical decomposition of isovaleraldehyde and di-n-propyl ketone. *J. Chem. Soc.* 1504-1511.

Flood, H. and **Förland, T.** 1947. The acid and basic properities of oxides. *Acta Chem. Scand.* 1: 592-603.

Flory, P.J. 1941. Molecular size distribution in three-dimensional polymers. I. Gelation. *J. Am. Chem. Soc.* 73: 3083-3100.

Fuoss, R.M. and **Mead, D.J.** 1943. Osmotic pressure of polyvinyl chloride solutions by a dynamic method. *J. Phys. Chem.* 47: 59-70.

Kirshenbaum, A.D. and **Grosse, A.V.** 1952. Determination of oxygen in metals and metal oxides by the isotopic method. *Trans. Am. Soc. Metals.* Preprint No. 39 pp. 1-11.

Lentz, C.W. 1964. Silicate minerals as sources of trimethylsilyl silicates and silicate structure analysis of sodium silicate solutions. *Inorganic Chem.* 3: 574-579.

Marcus, A.J., Marcus, S.N., Marcus, R. and **Watt, J.** 1989. Rapid production of ulcerative disease of the colon in newly weaned guinea-pigs by degraded carragenins. *J. Pharm. Pharmacol.* 41: 423-426.

Richardson, F.D. 1955. The vitreous state. Glass delegacy of the University of Sheffield, pp. 63-84.

Richardson, F.D. 1962. Nature of silicate slags - Carter memorial lecture. *J. Met. Club* pp. 21-31.

Sloman, H.A. 1959. Eight report on the heterogenity of steel ingots: section VI, London ISI, p. 45.

Toop, G.W. and **Samis, C.S.** 1962. Some new ionic concepts of silicate slags. *Can. Met. Q.* 1: 129-152.

Publications of C.R. Masson

Masson, C.R., Menzies, R.F., Cruickshank, J. and **Melville, H.W.** 1946. Bacterial cellulose for osmometer membranes. *Nature* (Lond.), 157: 74.

Melville, H.W. and **Masson, C.R.** 1948. Experimental methods in the osmometry of dilute rubber solutions. Proc. Second Rubber Tech. Conf., Heffer, Cambridge, pp. 152-161.

Masson, C.R. and **Melville, H.W.** 1949. Osmometry of high polymer solutions. I. Osmometer and membranes. *J. Polymer Sci.* 4: 323-336.

Masson, C.R. and **Melville, H.W.** 1949. Osmometry of high polymer solutions. II. Osmotic measurements using bacterial cellulose membranes. *J. Polymer Sci.* 4: 337-350.

Masson, C.R. and **Steacie, E.W.R.** 1950. Primary step in the mercury photo-sensitised decomposition of propane and of hydrogen. *J. Chem. Phys.* 18: 210-212.

Masson, C.R. and **Melville, H.W.** 1951. Modified osmotic balance. *J. Polymer Sci.* 6: 21-27.

Masson, C.R. and **Steacie, E.W.R.** 1951. The mercury photosensitised decomposition of methyl chloride. *J. Chem. Phys.* 19: 183-189.

Bosworth, P., Masson, C.R., Melville, H.W. and **Peaker, F.W.** 1952. Determination of the molecular weights of fractionated polyvinyl acetates by a new type of light-scattering instrument. *J. Polymer Sci.* 9: 565-574.

Masson, C.R. 1952. The photolysis of di-n-propylketone. *J. Amer. Chem. Soc.* 74: 4731-4735.

Masson, C.R. and **Caines, G.W.** 1954. Viscosity and molecular weight of degraded carrageenin. *Can. J. Chem.* 32: 51-58.

Whiteway, S.G. and **Masson, C.R.** 1954. Direct current power supply for AH-6 mercury arcs. *Can. J. Chem.* 32: 1154-1155.

Whiteway, S.G. and **Masson, C.R.** 1955. The carbon monoxide quantum yield in the photolysis of diisopropylketone. *J. Amer. Chem. Soc.* 77: 1508-1509.

Masson, C.R. 1955. The degradation of carrageenin. I. Kinetics in aqueous solution at pH 7. *Can. J. Chem.* 33: 597-603.

Masson, C.R. and **Goring, D.A.I.** 1955. Studies on carrageenin: the effect of sheer rate on viscosity. *Can. J. Chem.* 33: 895-903.

Masson, C.R., Santry, D. and **Caines, G.W.** 1955. The degradation of carrageenin. II. Influence of further variables. *Can. J. Chem.* 33: 1088-1096.

Whiteway, S.G. and **Masson, C.R.** 1956. Rate of association of n-propyl radicals. *J. Chem. Phys.* 25: 233-237.

Masson, C.R., Boekelheide, V. and **Noyes, W.A. Jnr.** 1956. Photochemical reactions. *Tech. Org. Chem.* 2: 258-384.

Turkdogan, E.T., Leake, L.E. and **Masson, C.R.** 1956. Thermodynamics of iron-carbon melts. *Acta Metall.* 4: 396-406.

Turkdogan, E.T. and **Masson, C.R.** 1958. Thermodynamics of iron-carbon metals. *Acta Metall.* 6: 519-520.

Masson, C.R. 1959. Slide-rule for converting percentages to mole fractions. *Anal. Chem.* 31: 1122-1124.

Masson, C.R. 1959. Preparative organische photochemie. *J. Amer. Chem. Soc.* 81: 3487.

Pearce, M.L. and **Masson, C.R.** 1960. The determination of oxygen in iron and steel. *Nature* (Lond.) 185: 373-374.

Pearce, M.L. and **Masson, C.R.** 1960. The determination of oxygen and nitrogen in iron and steel by an isotope-dilution method. *Iron Steel Inst.* London, Spec. Rep. 68: 121-132.

Whiteway, S.G., Coll-Palagos, M. and **Masson, C.R.** 1961. Slip casting magnesia. *Am. Ceram. Soc. Bull.* 40: 432-438.

Coll-Palagos, M. and **Masson, C.R.** 1961. Static and asymmetry potentials in cells with magnesia electrodes at high temperatures. *Trans. Farad. Soc.* 57: 1621-1626.

Masson, C.R. and **Pearce, M.L.** 1962. Determintion of nitrogen in iron and steel. Comparison of results obtained by the vacuum-fusion, Kjeldahl, and isotope dilution methods. *Trans. Metall. Soc.* AIME, 224: 1134-1139.

Jamieson, W.D. and **Masson, C.R.** 1962. Decarbonization of liquid iron-carbon alloys. *J. Amer. Chem. Soc.* 84: 4084-4089.

Masson, C.R. 1962. Written contribution to "Discussion on thermodynamics of slags". *J. Iron Steel Inst.* 200: 545.

Masson, C.R., Whiteway, S.G. and **Collings, C.A.** 1963. Slip casting calcium fluoride and lime-stabilized zirconia. *Am. Ceram. Soc. Bull.* 42: 745-747.

Burden, J.P. and **Masson, C.R.** 1964. Modified isotope dilution technique for the determination of oxygen in iron and steel. *J. Iron Steel Inst.* 202: 28-31.

Bell, H.B. and **Masson, C.R.** 1964. An improved high temperature cryoscopic technique. *J. Sci. Instrum.* 41: 389.

Masson, C.R. 1965. An approach to the problem of ionic distribution in liquid silicates. *Proc. Royal Soc.* A287: 201-221.

Peters, R.J.W., Masson, C.R. and **Whiteway, S.G.** 1965. Kinetics of desulphurization of liquid iron-sulphur alloys by mixtures of hydrogen with helium and argon. *Trans. Farad. Soc.* 61: 1745-1753.

Whiteway, S.G. and **Masson, C.R.** 1966. Effect of thermionic emission on electrochemical measurements at high temperatures. *Can. J. Chem.* 44: 2421-2427.

Masson, C.R., Whiteway, S.G., Jamieson, W.D. and **Collings, C.A.** 1966. Determination of oxygen in titanium by isotopic dilution. *Can., Metall. Q.* 5: 329-341.

Diaz, C., Masson, C.R. and **Richardson, F.D.** 1966. Electrochemical determination of oxygen in liquid silver. *Inst. Min. Metall. Trans.* Sect. C, 75: 183-186.

Masson, C.R. and **Whiteway, S.G.** 1967. Diffusion of oxygen in liquid silver by an electrochemical method. *Can. Metall. Q.* 6: 199-217.

Masson, C.R. 1967. Stable isotope-dilution techniques for the determination of gases in metals. *Metall. Rev.* 117: 147-168.

Masson, C.R. 1968. Ionic equilibria in liquid silicates. *J. Am. Ceram. Soc.* 51: 134-143.

Kojima, H., Whiteway, S.G. and **Masson, C.R.** 1968. Melting points of inorganic fluorides. *Can. J. Chem.* 46: 2968-2971.

Whiteway, S.G., Peters, R.J.W., Jamieson, W.D. and **Masson, C.R.** 1968. Decarburization of iron-carbon alloys by argon-oxygen mixtures. *Can. Metall. Q.* 7: 211-215.

Kojima, H. and **Mason, C.R.** 1969. Cryoscopic studies with molten fluorides. I. Depression of the freezing point of calcium fluoride by alkaline-earth fluorides and oxides and calcium silicates. *Can. J. Chem.* 47: 4221-4228.

Whiteway, S.G., Smith, I.B. and **Masson, C.R.** 1970. Theory of molecular size distribution in multichain polymers. *Can. J. Chem.* 48: 33-45.

Masson, C.R., Smith, I.B. and **Whiteway, S.G.** 1970. Molecular size distributions in multichain polymers: application of polymer theory to silicate melts. *Can. J. Chem.* 48: 201-202.

Masson, C.R., Smith, I.B. and **Whiteway, S.G.** 1970. Activities and ionic distributions in liquid silicates: Application of polymer theory. *Can. J. Chem.* 48: 1456-1464.

Wu, F.F.H., Gotz, J., Jamieson, W.D. and **Masson, C.R.** 1970. Determination of silicate anions by gas chromatographic separation and mass spectrometric identification of their trimethylsilyl derivatives. *J. Chromatog.* 48: 515-520.

Caines, G.W. and **Mason, C.R.** 1970. Flow of bunker C oil and oil-water emulsion on solid surfaces. Atlantic Regional Laboratory Report No. 4.

Smith, I.B. and **Masson, C.R.** 1970. Activity-composition relationships of cobalt in 80% platinum-20% rhodium alloy. *Can. Metall. Q.* 9: 415-418.

Caines, G.W., Robson, D.R. and **Masson, C.R.** 1970. Effect of water on the ignition temperature of bunker C oil. Atlantic Regional Laboratory Report No. 7.

Gotz, J. and **Masson, C.R.** 1970. Trimethylsilyl derivatives for the study of silicate structures. Part I. A direct method of trimethylsilylation. *J. Chem. Soc.* (A), 2683-2686.

Masson, C.R. 1970. Thermodynamic properties of $PbO\text{-}SiO_2$ slags by emf measurements. *Can. Metall. Q.* 9: 563.

Masson, C.R. and **Whiteway, S.G.** 1970. Discussion of "Electrochemical measurement of oxygen diffusivity in liquid silver". *Metall. Trans.* 1: 2333.

Smith, I.B. and **Masson, C.R.** 1971. Activities and ionic distributions in cobalt silicate melts. *Can. J. Chem.* 49: 683-690.

Gotz, J. and **Masson, C.R.** 1971. Trimethylsilyl derivates for the study of silicate structures. Part II. Orthosilicate, pyrosilicate and ring structures. *J. Chem. Soc.* (A), 686-688.

Distin, P.A., Whiteway, S.G. and **Masson, C.R.** 1971. Solubility of oxygen in liquid iron from 1785° to 1961°C. A new technique for the study of slag-metal equilibria. *Can. Metall. Q.* 10: 13-18.

Distin, P.A., Whiteway, S.G. and **Masson, C.R.** 1971. Thermodynamics and constitution of ferrous silicate melts. *Can. Metall. Q.* 10: 73-78.

Masson, C.R., Gotz, J., Jamieson, W.D., McLachlan, J.L. and **Volborth, A.** 1971. Chromatographic and mineralogical study of lunar fines and glass. In Proc. Second Lunar Science Conf., *Geochim. Cosmochim. Acta,* Suppl. 2, Vol. 1. pp. 957-971.

Gotz, J., Jamieson, W.D. and **Masson, C.R.** 1971. Trimethylsilyl derivatives of glass constituents: a study of Lentz technique as applied to the constitution of silicate melts and glasses. In Proc. Ann Meeting Int. Comm. Glass, Eds. S. Bateson and A.G. Sadler, *Can. Ceram. Soc.* pp. 69-74.

Gotz, J. and **Masson, C.R.** 1971. Trimethylsilylation of silica anions: a method of studying the structure of crystalline silicates and glasses. *Int. Congr. Glass Tech. Commun.,* 9th, 1: 261-276.

Masson, C.R., Smith, I.B. and **Whiteway, S.G.** 1971. Reply to Gordon and Judd. *Nature* (Lond.), 234: 97-98.

Gotz, J., Masson, C.R., and **Castelliz, L.M.** 1972. Crystallization of lead silicate glass as studied by trimethylsilylation and chromatographic separation of the anionic constituents. In *Amorphous materials,* Eds., R.W. Douglas and B. Ellis, Wiley-Interscience, New York, pp. 317-325.

Masson, C.R., Smith, I.B., Jamieson, W.D., McLachlan, J.L. and **Volborth, A.** 1972. Chromatograpic and mineralogical study of Apollo 14 fines. In Proc. Third Lunar Science Conference, *Geochim. Cosmochim. Acta,* Vol. 1, pp. 1029-1036.

Grant, R. and **Masson, C.R.** 1972. Viscosity of sodium silicate solutions. *J. Colloid Interface Sci.* 41: 606-607.

Cradwick, P.D.G., Farmer, V.C., Russell, J.D., Masson, C.R., Wada, K. and **Yoshinaga, N.** 1972. Imogolite, a hydrated aluminum silicate of tubular structure. *Nature* (Lond.) Phys. Sci. 240: 187-189.

Masson, C.R. 1972. Thermodynamics and constitution of silicate slags. *J. Iron and Steel Inst.* 210: 89-96.

Whiteway, S.G., Smith, I.B. and **Masson, C.R.** 1973. Theory of molecular size distribution in multichain polymers: condensation of R-A_f with R′-B_f, and R-A_f with R′-$A_{f'}$. *Can. J. Chem.* 51: 1422-1431.

Sharma, S.K., Dent-Glasser, L.S. and **Masson, C.R.** 1973. Trimethylsilyl derivatives for the study of silicate structures. Part III. Sodium silicate hydrates. *J. Chem. Soc., Dalton Trans.* 1324-1328.

Masson, C.R., Jamieson, W.D. and **Mason, F.G.** 1974. *Ionic constitution of metallurgical slags.* In Physical chemistry of process metallurgy: The Richardson Conference. Inst. Mining and Metal., London, pp. 223-231.

Masson, C.R. 1974. Polymer theory of silicate melts. *Chem. Technol.* 56-62.

Masson, C.R. and **Whiteway, S.G.** 1974. Reply to comment: on the Whiteway-Smith-Masson and Flory-Stockmayer theories of polymerization. *Can. J. Chem.* 52: 1190-1191.

Caley, W.F. and **Masson, C.R.** 1975. *Thermodynamics of lead phosphate slags by an electrochemical method.* In Metal-slag-gas reactions and processes, Eds Z.A. Foroulis and W.W. Smeltzer, The Electrochemical Soc., pp. 140-154.

Sakawa, M., Whiteway, S.G. and **Masson, C.R.** 1976. Activities of FeO in the ternary system SiO_2-MgO-FeO and constitution of SiO_2. *Tetsu to Hagane,* 62: 176-181.

Grau, A.E., Caley, W.F. and **Masson, C.R.** 1976. Thermodynamics of lead silico-fluoride melts. *Can. Metall. Q.* 15: 267-273.

Caley, W.F. and **Masson, C.R.** 1976. Activities and oxygen transport in PbO-SiO_2-P_2O_5 melts. *Can. Metall. Q.* 15: 359-365.

Grau, A.E. and **Masson, C.R.** 1976. Densities and molar volumes of silicate melts. *Can. Metall. Q.* 15: 367-374.

Masson, C.R. 1977. *Anionic constitution of glass-forming melts.* In Glass '77, Proc. XIth Int. Cong. on Glass, Prague, Ed J. Gotz, CVTS-DUM Techniky, pp. 1-41.

Grau, A.E. and **Masson, C.R.** 1977. Solubility of oxygen in PbO-SiO_2 melts. *Can. Metall. Q.* 16: 82-87.

Masson, C.R. 1977. Physical and High Temperature Chemistry. In ARL, the first 25 years. Ed. J.S. MacRae, Staff Association Atlantic Regional Laboratory, Halifax.

Gotz, J. and **Masson, C.R.** 1978. Trimethylsilyl derivatives for the study of silicate structures. Part 4. The conversion of hemimorphite into willemite. *J. Chem. Soc. Dalton Trans.* 1134-1138.

Calhoun, H.P. and **Masson, C.R.** 1978. Trimethylgermyl derivatives for the study of silicate structures. *J. Chem. Soc. Chem. Commun.* 560-561.

Calhoun, H.P. and **Masson, C.R.** 1978. Trimethylsilyl derivatives for the study of silicate structures. Part 5. Trimethylsilylation of dioptase. *J. Chem. Soc. Dalton Trans.* 1342-1349.

Jamieson, W.D., Calhoun, H.P. and **Masson, C.R.** 1978. Mass spectrometry of trimethylsilyl ethers of complex silicate and fluorosilicate anions. 26th Annual conference on mass spectrometry and allied topics. St. Louis, pp. 247-249.

Masson, C.R. and **Caley, W.F.** 1978. Thermodynamics and constitution of silicate melts. The system $MO+MF_2+SiO_2$. *J. Chem. Soc. Faraday Trans. I,* 74: 2942-2951.

Caley, W.F. and **Masson, C.R.** 1978. Thermodynamics and constitution of silicate melts. The system $PbO+PbF_2+SiO_2$. *J. Chem. Soc. Faraday Trans. I,* 74: 2952-2967.

Calhoun, H.P., Jamieson, W.D. and **Masson, C.R.** 1979. Trimethylsilyl derivates for the study of silicate structures. Part 6. Identification of fluorosilicate ions from a lead (II) Oxide-lead (II) fluoride-silicon (IV) oxide (3/1/1) glass. *J. Chem. Soc. Dalton Trans.* 454-459.

Masson, C.R. 1980. Summary and comments (session A-E). In Proceedings, Direction of coal research in Eastern Canada. Atlantic Research Laboratory Technical Report, No. 25, pp. 143-148.

Calhoun, H.P., and **Masson, C.R.** 1980. Trimethylsilyl derivates for the study of silicate structures. Part 7. Calcium silicate structures. *J. Chem. Soc. Dalton Trans.* 1282-1291.

Calhoun, H.P., Masson, C.R. and **Jansen, M.** 1980. Trimethylsilylation of $Ag_{10}Si_4O_{13}$: cyclisation of the linear chain anion $Si_4O_{13}^{-10}$. *J. Chem. Soc. Chem. Commun.* 576-577.

Masson, C.R. 1981. Foreword: International Symposium on Metallurgical slags. *Can. Metall. Q.* 20: 1-2.

Calhoun, H.P. and **Masson, C.R.** 1981. *Studies on silicate structures by the method of trimethylsilylation.* In Rev. Silicon, Germanium, Tin and Lead Compd. Ed. M. Gielen, 5, No. 4: 153-243.

Masson, C.R. 1981. Application of Temkin's law to polyionic melts. *Can. Metall. Q.* 20: 27-29.

Caley, W.F., Marple, B.R. and **Masson, C.R.** 1981. Diffusion and electrochemical transport of oxygen in $PbO-SiO_2$ melts. *Can. Metall. Q.* 20: 213-223.

Dent-Glasser, L.S., Lachowski, E.E., Qureshi, M.Y., Calhoun, H.P., Embree, D.J., Jamieson, W.D. and **Masson, C.R.** 1981. Identification of some of the polysilicate components of trimethylsilylated cement paste. *Cem. Concr. Res.* 11; 775-780.

Leewis, K.G., Caley, W.F. and **Masson, C.R.** 1982. Electrochemical transport of sulphur in $CaO-Al_2O_3-SiO_2$ slags. In Proc. Int. Symp. on the physical chemistry of iron and steel making, Toronto, (C.I.M.).

Shinmei, M., Imai, T., Yokokawa, T. and **Masson, C.R.** 1984. *Study of gas species above fluoride-containing silicate melts.* In Proc. 1st. Int. Symp. on molten salt chemistry and technology, Electrochemical Soc. of Japan, Kyoto.

Leewis, K.G., Caley, W.F. and **Masson, C.R.** 1984. *Electrochemically-enhanced oxygen transfer and desulphurization in a gas-slag-metal system.* In Proc. 2and. Int. Symp. on metallurgical slags and fluxes, Eds. H.A. Fine and D.R. Gaskell, Metallurgical Soc. AIME pp. 685-697.

Masson, C.R. 1984. *The chemistry of slags - an overview.* In Proc., 2and., Int. Symp. on metallurgical slags and fluxes, Eds. H.A. Fine and D.R. Gaskell, Metallurgical Soc. AIME, pp. 3-44.

Dou, S., Pacey, P.D., Masson, C.R. and **Marple, B.R.** 1985. Grain growth in CaO-stabilised ZrO_2 in the presence of a liquid phase. *J. Am. Ceram. Soc.* 68: C80-C81.

Dou, S., Masson, C.R. and **Pacey, P.D.** 1985. Mechanism of oxygen permeation through lime-stabilized zirconia. *J. Electrochem. Soc.* 132: 1843-1849.

PROCEEDINGS OF MEETINGS
1987-88
HALIFAX CHAPTER

1st Ordinary Meeting, 14 October, 1987
Pattern and Non-pattern in the History of Life, Stephen Jay Gould, Harvard University.

2nd Ordinary Meeting, 2 November, 1987
Recent Dinosaur Finds in Nova Scotia and their Geological History, Robert Grantham, Nova Scotia Museum.

3rd Ordinary Meeting, 7 December, 1987
Studies on the Effects of Acid Precipitation in Atlantic Salmon in Western Nova Scotia, Harry Freeman, Department of Fisheries and Oceans.

4th Ordinary Meeting, 18 January, 1988
High-Temperature Superconductors, David Tindall, Dalhousie University.

5th Ordinary Meeting, 1 February, 1988
The Application of Marine Acoustics in Fish Stock Assessment, Lloyd Dickie, Bedford Institute of Oceanography.

6th Ordinary Meeting, 7 March, 1988
Applied Microelectronics, Douglas Pincock, Applied Microelectronics Institute.

7th Ordinary Meeting, 3 April, 1988
Computer Communications and Networks, Peter Jones, Dalhousie University.

127th Annual General Meeting, 5 May, 1988
Science, Technology and the Future of Canada, Kelvin Ogilvie, Acadia University.

VALLEY CHAPTER

1st Ordinary Meeting
The Direct Effects of Changing Carbon Dioxide Concentrations on Plant Growth, E. Reekie, Acadia University.

2nd Ordinary Meeting
Objectives and Present Activities of the Food Technology Centre, Richard Ablett, P.E.I. Food Technology Centre.

3rd Ordinary Meeting
A Pictorial Essay on Agriculture in New Zealand and Australia, Fred Walsh, Nova Scotia Department of Agriculture and Marketing.

4th Ordinary Meeting
Fiddleheads as a Horticultural Crop, Robert Prange, Agriculture Canada.

5th Ordinary Meeting
Pseudomonas—a Plant's Best Friend, Colin Bell, Acadia University.

THE PRESIDENT'S REPORT

The past year got off to an excellent start with the Stephen Jay Gould lecture "Pattern and Non-Pattern in the History of Life" commemorating the 125th anniversary of the Institute. Approximately 1100 people were in attendance, making it, as far as we know, the largest meeting ever held by the Institute. A special expression of thanks is extended to Dr. Donald Gordon, who was instrumental in arranging Dr. Gould's visit.

Extra effort was made during the year to increase membership which grew by approximately 10 per cent to a total of about 225. This was encouraging but attendance at meetings was somewhat disappointing, ranging from a low of 16 to a high of 53, excluding the anniversary lecture. There has been general agreement that a member of the Council should be designated as public relations officer next year to stimulate attendance at meetings.

Speakers during the year covered a range of topics related to current scientific endeavours in Nova Scotia. Following Dr. Gould's lecture in October, the lectures covered:

- November: "Recent Dinosaur Finds in Nova Scotia and their Geological History", Bob Grantham, Nova Scotia Museum
- December: "Studies on the Effects of Acid Precipitation in Atlantic Salmon in Western Nova Scotia", Harry Freeman, Fisheries and Oceans.
- January: "High-Temperature Superconductors", David Tindall, Dalhousie University.
- February: "The Application of Marine Acoustics in Fish Stock Assessment", Lloyd Dickie, Bedford Institute of Oceanography.
- March: "Applied Microelectronics", Douglas Pincock, Applied Microelectronics Institute.
- April: "Computer Communications and Networks", Peter Jones, Dalhousie University.

The public lecture tonight on "Science, Technology and the Future of Canada" by Dr. Kelvin Ogilvie, Vice-President of Acadia University, will be an appropriate closing for the season. The speaker's program for the coming year will start off with a lecture on domoic acid in mussels, followed by others including undergraduate science education, developments in dental science, oceanography and astronomy.

The Institute continues to be financially sound and provides a forum for the promotion of scientific education and research. World-wide distribution of the Proceedings was maintained and the Institute acknowledges with grateful thanks financial support for the Proceedings from Acadia University, the Nova Scotia Museum, Technical University of Nova Scotia and the Natural Sciences and Engineering Research Council of Canada.

We were saddened during the year by the loss of two of our longstanding members, Pat Wyman and Charles Masson. Dr. Masson was serving as Treasurer at the time and his passing was particularly felt by many. This prompted the Council to set up a Memorial Fund in memory of Institute members to be used to support special events promoting the activities of the Institute.

To conclude, it was a pleasure to serve as President of the Institute during this special year and I extend my thanks to the various members of the Executive and Council for their interest, hard work and support. I also offer special thanks to John Walter for stepping in as Interim Treasurer and to Alan Taylor for his work as Editor of Proceedings.

E. Graham Bligh

INSTRUCTIONS TO AUTHORS

The *Proceedings of the Nova Scotian Institute of Science* publishes the proceedings of the Institute and original articles, including notes, pertaining to the natural sciences of the Atlantic Provinces, especially in biology and geology. Manuscripts may be either in English or French, and acceptance for publication is based on recommendations of referees.
Manuscripts should be typewritten, double-spaced on white paper 21.5 by 28 cm (8.5 by 11 in) with margins of 4 cm (1.5 in). Underline only material to be set in italics, and use capital letters only when letter or words should appear in capitals in the printed paper. Layout and style should follow that used in this volume. The original copy and one duplicate are required. Each page of the manuscript should be numbered, the first page carrying only the title, authors' names and affiliations, and any necessary footnotes.

Spelling should follow that of **Webster's Third New International Dictionary**, and authors are responsible for consistency in spelling.

Abbreviations, nomenclature, and symbols for units of measurements should follow international recommendations. Metric units, SI units and decimals should be used whenever possible. Use day/month/year sequence for dates. Do not use periods after such abbreviations as "mm, kg, DOE, NRC, etc."

Taxonomic keys should be in aligned-couplet form in zoology and paleontology, and in multi-level indent form in botany. Synonomy in botany, zoology, and paleontology should be in the short form—taxon, author, year, page—with full citation in the references.

An abstract of not more than 200 words should precede the body of the text. This should be followed by an introduction, methods, results or observations, discussion, and references. In some cases a combination of these sections may be more effective.

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Author, A.N. and Other, T.H.E. 1978. Title of the article. *Name of Journal,* (Volume) 99: 1-10. (Omit word volume)
Author, A.N. and Coauthor, A. 1978. *Full Title of Book.* Publisher, City.
Author, A. 1978. Title of Article. In *Title of Book or Symposium Volume.* (ed. J. Smith and B. Jones). Publisher, City, pp. 256-301.

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